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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

**TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371**

003300-920

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)

unassigned **10/088639**

INTERNATIONAL APPLICATION NO.
PCT/SE00/02082

INTERNATIONAL FILING DATE
26 October 2000

PRIORITY DATE CLAIMED
28 October 1999

TITLE OF INVENTION
NOVEL COMPOUNDS

APPLICANT(S) FOR DO/EO/US

THOMAS BRODIN, PIA J. KARLSTRÖM, LENNART G. OHLSSON, JESPER M. TORDSSON, PHILIP P. KEARNEY
and BO H. K. NILSON

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:


1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information: A certified copy of Swedish Application No. 9903895-2, filed 28 October 1999, was submitted during the international phase of examination. Thus the claim for priority has been perfected.



21839

U.S. APPLICATION NO. (If known, give 37 C.F.R. 1.51(a) serial number) unassigned		INTERNATIONAL APPLICATION NO. PCT/SE00/02082		ATTORNEY'S DOCKET NUMBER 003300-920	
21. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE and International Search Report not prepared by the EPO or JPO \$1,040.00 (960) International preliminary examination fee (37 CFR 1.482) not paid to U.S. PATENT AND TRADEMARK OFFICE but International Search Report prepared by the EPO or JPO \$890.00 (970) International preliminary examination fee (37 CFR 1.482) not paid to U.S. PATENT AND TRADEMARK OFFICE but international search fee (37 CFR 1.445(a)(2)) paid to U.S. PATENT AND TRADEMARK OFFICE \$740.00 (958) International preliminary examination fee (37 CFR 1.482) paid to U.S. PATENT AND TRADEMARK OFFICE but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$710.00 (956) International preliminary examination fee (37 CFR 1.482) paid to U.S. PATENT AND TRADEMARK OFFICE and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 (962)					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$ 1,040.00	
Surcharge of \$130.00 (154) for furnishing the oath or declaration later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	57 -20 =	37	X\$18.00 (966)	\$ 666.00	
Independent Claims	1 -3 =	0	X\$84.00 (964)	\$ --	
Multiple dependent claim(s) (if applicable)			+ \$280.00 (968)	\$	
TOTAL OF ABOVE CALCULATIONS =				\$ 1,706.00	
Reduction for 1/2 for filing by small entity, if applicable (see below).				+	\$ --
SUBTOTAL =				\$ 1,706.00	
Processing fee of \$130.00 (156) for furnishing the English translation later than 20 <input type="checkbox"/> 30 <input type="checkbox"/> months from the earliest claimed priority date (37 CFR 1.492(h)).				+	\$ --
TOTAL NATIONAL FEE =				\$ 1,706.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 (581) per property				+	\$ 40.00
TOTAL FEES ENCLOSED =				\$ 1,746.00	
				Amount to be refunded:	\$
				charged:	\$
a. <input type="checkbox"/> Small entity status is hereby claimed. b. <input checked="" type="checkbox"/> A check in the amount of \$ <u>1,746.00</u> to cover the above fees is enclosed. c. <input type="checkbox"/> Please charge my Deposit Account No. <u>02-4800</u> in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. d. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4800</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Benton S. Duffett, Jr. BURNS, DOANE, SWECKER & MATHIS, L.L.P. P.O. Box 1404 Alexandria, Virginia 22313-1404 (703) 836-6620					
				 SIGNATURE Benton S. Duffett, Jr. NAME	
				22,030 REGISTRATION NUMBER March 20, 2002 DATE	



100330039 032002

#6

Patent
Attorney's Docket No. 003300-920

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
)	
Thomas BRODIN <i>et al.</i>)	Group Art Unit: Not yet assigned
)	
Serial No.: 10/088,639)	Examiner: Not yet assigned
)	
Filed: March 20, 2002)	Confirmation No.: 7152
)	
For: NOVEL COMPOUNDS)	ATTENTION: BOX SEQUENCE

REPLY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification of Defective Response dated August 20, 2002, please
amend the above-identified application as follows:

IN THE SPECIFICATION:

In compliance with 37 C.F.R. § 1.823(a), please insert the attached paper copy of the
"Sequence Listing" after the last page of the above-identified application to replace the
Sequence Listing previously filed on July 19, 2002.

Patent
Serial No.: 10/088,639
Attorney's Docket No. 003300-920

REMARKS

The paper copy of the Sequence Listing for the subject application, is by this amendment, added after the last page of the application to replace the Sequence Listing previously filed on July 19, 2002.

Favorable consideration on the merits is respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By Deborah H. Yellin
Deborah H. Yellin
Registration No. 45,904

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: September 16, 2002

Patent
Attorney's Docket No. 003300-920

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
)	
Thomas Brodin <i>et al.</i>)	Group Art Unit: Not yet assigned
)	
Serial No.: 10/088,639)	Examiner: Not yet assigned
)	
Filed: March 20, 2002)	ATTENTION: BOX SEQUENCE
)	
For: NOVEL COMPOUNDS)	Confirmation No.: 7152

REPLY

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

In response to the Notification of Missing Requirements Under 35 U.S.C.

371 dated May 20, 2002, please amend the above-identified application as follows:

IN THE SPECIFICATION:

In compliance with 37 C.F.R. §1.823(a), please insert the attached paper copy of the "Sequence Listing" after the last page of the above-identified application to replace the Sequence Listing identified on pages 1-22 after the Figures.

Patent
Attorney's Docket No. 003300-920

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
)	
Thomas Brodin <i>et al.</i>)	Group Art Unit: Not yet assigned
)	
Serial No.: 10/088,639)	Examiner: Not yet assigned
)	
Filed: March 20, 2002)	ATTENTION: BOX SEQUENCE
)	
For: NOVEL COMPOUNDS)	Confirmation No. 7152

DECLARATION PURSUANT TO
37 C.F.R. §§1.821-1.825

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:


I, Deborah H. Yellin, declare as follows:

1. That the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(c) and (e), respectively, are the same in compliance with §1.821(f).
2. That the submission, filed in accordance with 37 C.F.R. §1.821(g)[or (h)], herein does not include new matter [or go beyond the disclosure in the international application].
3. That the substitute copy of the computer readable form, submitted in accordance with 37 C.F.R. §1.825(d), is identical to that originally filed.

Serial No.: 10/088,639
Atty. Dkt. No. 003300-920

I hereby declare that all statements made herein of my own knowledge are true and that all statements were made on information and belief and are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

July 19, 2002
Date


Deborah H. Yellin
Registration No. 45,904

Patent
Attorney's Docket No. 003300-920

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	BOX PCT
)	
THOMAS BRODIN et al.)	Attention: DO/EO/US
)	
Application No.: (Unassigned))	Group Art Unit: (unassigned)
)	
Filed: March 20, 2002)	Examiner: (unassigned)
)	
For: NOVEL COMPOUNDS)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

This is a national phase filing of International Application No. PCT/SE00/02082,
filed October 26, 2000.

Please amend the Application as indicated.

IN THE ABSTRACT:

Please add the Abstract of the Disclosure that is provided on a separate sheet.

IN THE CLAIMS:

Kindly replace Claim 17, 22 to 24, 28 to 30, 32 to 40, 45 to 48, 51 and 52
as follows:

17. (Amended) A target structure displayed in, or on the surface of, tumour
cells, said target structure

- b) being displayed in, and on the surface of, human gastrointestinal epithelial cells,
- c) having substantial homology with $\alpha 6$ and/or $\beta 4$ integrin chains or variants thereof, representing a shared or unique epitope,
- d) being highly expressed on the surface of tumour cells, and
- e) being a target for cytotoxic effector mechanisms.

23. (Amended) A target structure according to claim 17, the expression of which is correlated to gastrointestinal epithelial differentiation.

24. (Amended) A target structure according to claim 17, which comprises essentially the amino acid sequence of $\alpha 6$ integrin shown in SEQ ID NO: 3 and/or of $\beta 4$ integrin shown in SEQ ID NO: 4, and/or one or more fragments, and/or variants or splice variants, and or subunits, thereof.

29. (Amended) A substance which binds to the target structure as defined in claim 17, which substance is an organic chemical molecule or a peptide.

30. (Amended) A substance, which is an anti-idiotypic of a binding structure to said target structure as defined in claim 17.

32. (Amended) A substance which blocks the function of the target structure as defined in claim 17, which substance is an organic chemical molecule or a peptide.

33. (Amended) A binding structure which recognizes a target structure as defined in claim 17, and which is of an organic chemical nature.

34. (Amended) A pharmaceutical composition comprising as an active principle an antibody as defined in claim 1.

35. (Amended) A pharmaceutical composition comprising as an active principle a target structure as defined in claim 17.

36. (Amended) A pharmaceutical composition comprising as an active principle a substance as defined in claim 29.

37. (Amended) A vaccin composition comprising as an active principle an antibody as defined in claim 1.

38. (Amended) A method of therapy for treating conditions based on an anti-angiogenic mechanism, whereby an antibody as defined in claim 1 is administered to a human subject.

39. (Amended) A method of treating human metastatic diseases, wherein an antibody as defined in claim 1 is administered to a human subject.

40. (Amended) A method of in vitro histopathological diagnosis and prognosis of human malignant disease, whereby a sample is contacted with an antibody as defined in claim 1 and an indicator.

45. (Amended) A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of an antigen comprising a target structure as defined in claim 17.

46. (Amended) A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of an antibody as defined in claim 1 is assayed.

47. (Amended) A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of a complex of a) an antigen comprising a target structure, as defined in claim 17 is assayed, and b) an antibody, or a derivative or a fragment thereof, having a binding structure for a target structure displayed in, and on the cell surface of, human gastrointestinal epithelial tumour cells and in a subpopulation of normal human gastrointestinal epithelial cells, said binding structure comprising the complementarity determining region (CDR) sequences in the light chain comprising essentially the amino acids number 23-33 (CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, and the CDR sequences in the heavy chain comprising essentially the amino acids number 158-162 (CDR1), 177-193

(CDR2), 226-238 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties, is assayed.

48. (Amended) A method for in vivo diagnosis and prognosis of human malignant disease, whereby the localisation of an antibody, as defined in claim 1, to tumour deposits in a human subject is determined.

51. (Amended) A method according to claim 48 which is quantitative.

52. (Amended) A method for therapy of human malignant disease, whereby an antibody, as defined in claim 1, is administered to a human subject.

Please add the following new Claims 53 to 57.

53. (New) A vaccin composition comprising as an active principle a target structure as defined in claim 17.

54. (New) A vaccin composition comprising as an active principle a substance as defined in claim 29.

56. (New) A method of therapy for treating conditions based on an anti-angiogenic mechanism, whereby a substance as defined in claim 29 is administered to a human subject.

57. (New) A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of a complex of a) a target structure, as defined in claim 29, is assayed, and b) an antibody or a derivative or a fragment thereof, having a binding structure for a target structure displayed in, and on the cell surface of, human gastrointestinal epithelial tumour cells and in a subpopulation of normal human gastrointestinal epithelial cells, said binding structure comprising the complementarity determining region (CDR) sequences in the light chain comprising essentially the amino acids number 23-33 (CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, and the CDR sequences in the heavy chain comprising essentially the amino acids number 158-162 (CDR1), 177-193 (CDR2), 226-238 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties, is assayed.

Application No. (Unassigned)
Attorney's Docket No. 003300-920
Page 8

REMARKS


The present Amendment modifies the claim format only so as to eliminate the use of multiple dependency.

An Information Disclosure Statement is being filed concurrently herewith.

The examination and allowance of the Application are respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: 
Benton S. Duffett, Jr.
Registration No. 22,030

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: March 20, 2002

Application No. (Unassigned)
Attorney's Docket No. 003300-920
Mark-up of Abstract - Page 1 of 1

Attachment to Preliminary Amendment dated March 20, 2002

Abstract of the Disclosure

An antibody, or a derivate or a fragment thereof, having a binding structure for a target structure is described. The antibody is displayed in, and on the cell surface of, human gastrointestinal epithelial tumour cells and in a subpopulation of normal human gastrointestinal epithelial cells. Said binding structure comprises the complementarity determining region (CDR) sequences in the light chain comprising essentially the amino acids number 23-33 (CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, and the CDR sequences in the heavy chain comprising essentially the amino acids number 158-162 (CDR1), 177-193 (CDR2), 226-238 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties. There is also described a target structure displayed in, or on the surface of tumour cells, vaccine compositions, pharmaceutical compositions as well as methods related to human malignant diseases.

Application No. (Unassigned)
Attorney's Docket No. 003300-920
Mark-up of Claims - Page 1 of 6

Attachment to Preliminary Amendment dated March 20, 2002
Mark-Up Copy of Claims 17, 22 to 24, 28 to 30, 32 to 40, 45 to 48, 51 and 52

17. (Amended) A target structure displayed in, or on the surface of, tumour cells, said target structure

a) having the ability of being specifically blocked by and to specifically block the binding structure of an antibody as defined in [any one of claims 1-14] claim 1, and other binding structures with similar binding properties,

b) being displayed in, and on the surface of, human gastrointestinal epithelial cells,

c) having substantial homology with $\alpha 6$ and/or $\beta 4$ integrin chains or variants thereof, representing a shared or unique epitope,

d) being highly expressed on the surface of tumour cells, and

e) being a target for cytotoxic effector mechanisms.

22. (Amended) A target structure according to [any one of claims 17-21] claim 17, which is expressed homogenously in human colonic epithelial cells and less in pancreatic duct and bile duct cells.

23. (Amended) A target structure according to [any one of claims 17-22] claim 17, the expression of which is correlated to gastrointestinal epithelial differentiation.

24. (Amended) A target structure according to [any one of claims 17-23] claim 17, which comprises essentially the amino acid sequence of $\alpha 6$ integrin shown in SEQ ID

Application No. (Unassigned)
Attorney's Docket No. 003300-920
Mark-up of Claims - Page 2 of 6

Attachment to Preliminary Amendment dated March 20, 2002

Mark-Up Copy of Claims 17, 22 to 24, 28 to 30, 32 to 40, 45 to 48, 51 and 52

NO: 3 and/or of β 4 integrin shown in SEQ ID NO: 4, and/or one or more fragments, and/or variants or splice variants, and or subunits, thereof.

28. (Amended) A target structure according to [any one of claims 24-27] claim 24 recognised, exclusively or not, in its non-reduced form by the binding structure comprised by the antibody [as defined in any one of claims 1-16], or a derivative or a fragment thereof, having a binding structure for a target structure displayed in, and on the cell surface of, human gastrointestinal epithelial tumour cells and in a subpopulation of normal human gastrointestinal epithelial cells, said binding structure comprising the complementarity determining region (CDR) sequences in the light chain comprising essentially the amino acids number 23-33 (CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, and the CDR sequences in the heavy chain comprising essentially the amino acids number 158-162 (CDR1), 177-193 (CDR2), 226-238 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties.

29. (Amended) A substance which binds to the target structure as defined in [any one of claims 17-28] claim 17, which substance is an organic chemical molecule or a peptide.

Application No. (Unassigned)
Attorney's Docket No. 003300-920
Mark-up of Claims - Page 3 of 6

Attachment to Preliminary Amendment dated March 20, 2002
Mark-Up Copy of Claims 17, 22 to 24, 28 to 30, 32 to 40, 45 to 48, 51 and 52

30. (Amended) A substance, which is an anti-idiotypic of a binding structure to said target structure as defined in [any one of claims 17-28] claim 17.

32. (Amended) A substance which blocks the function of the target structure as defined in [any one of claims 17-28] claim 17, which substance is an organic chemical molecule or a peptide.

33. (Amended) A binding structure which recognizes a target structure as defined in [any one of claims 17-28] claim 17, and which is of an organic chemical nature.

34. (Amended) A pharmaceutical composition comprising as an active principle an antibody as defined in [any one of claims 1-16] claim 1.

35. (Amended) A pharmaceutical composition comprising as an active principle a target structure as defined in [any one of claims 17-28] claim 17.

36. (Amended) A pharmaceutical composition comprising as an active principle a substance as defined in [any one of claims 29-32] claim 29.

Attachment to Preliminary Amendment dated March 20, 2002
Mark-Up Copy of Claims 17, 22 to 24, 28 to 30, 32 to 40, 45 to 48, 51 and 52

Application No. (Unassigned)
Attorney's Docket No. 003300-920
Mark-up of Claims - Page 5 of 6

Attachment to Preliminary Amendment dated March 20, 2002
Mark-Up Copy of Claims 17, 22 to 24, 28 to 30, 32 to 40, 45 to 48, 51 and 52

46. (Amended) A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of an antibody as defined in [any one of claims 1-16] claim 1 is assayed.

47. (Amended) A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of a complex of a) an antigen comprising a target structure, as defined in [any one of claims 17-28, or a structure, as defined in any one of claims 29-32,] claim 17 is assayed, and b) an antibody, [as defined in any one of claims 1-16,] or a derivative or a fragment thereof, having a binding structure for a target structure displayed in, and on the cell surface of, human gastrointestinal epithelial tumour cells and in a subpopulation of normal human gastrointestinal epithelial cells, said binding structure comprising the complementarity determining region (CDR) sequences in the light chain comprising essentially the amino acids number 23-33 (CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, and the CDR sequences in the heavy chain comprising essentially the amino acids number 158-162 (CDR1), 177-193 (CDR2), 226-238 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties, is assayed.

10/p.17

NOVEL COMPOUNDS

The present invention is related to an antibody, or a derivate, or a fragment thereof, having a binding structure for a target structure displayed in, and on the cell surface of, human gastrointestinal epithelial tumour cells and in a subpopulation of normal human gastrointestinal epithelial cells; and to a target structure displayed in, or on the surface of tumour cells; vaccine compositions; pharmaceutical compositions; as well as methods related to human malignant diseases.

10 BACKGROUND OF THE INVENTION

Surgery is the primary treatment of colorectal cancer leading to five-year survival rates of 90 to 40 percent depending on the state of tumour progression from Dukes Stage A to C. Conventional adjuvant therapy that includes radiation therapy and chemotherapy has been able to reduce the death rates further by approximately 30 percent (1). Despite these achievements cancer of the colon and rectum is one of the major causes of death in human cancer. Immunological therapy has been extensively attempted. However, colon cancer has generally been resistant to immunotherapy and is considered to be of low immunogenicity. Patients with colon cancer neither respond to IL-2 treatment or adoptive transfer of in vitro cultured tumour infiltrating lymphocytes otherwise active in patients with immunogenic malignancies such as melanoma. Most encouraging however, Riethmüller et al. reported a 32 percent decreased seven-year death rate for Dukes Stage C colorectal cancer treated after primary tumour resection with a naked murine mAb directed to a tumour and normal epithelial associated antigen (Ep-CAM) (2), indicating that other immunotherapeutic modalities could be effective.

A significant improvement of adjuvant immunotherapy and of the treatment of more advanced stages of cancer

should require a more potent effector mechanism than provided by a naked mAb. In principle, an increased potency should require an increased tumour selectivity of the targeting antibody.

5 The limited number of colon cancer associated antigens defined today have been discovered using hybridoma produced murine mAbs resulting from xenogenic immunisations with human tumours (3).

10 The use of large phage display libraries for the identification of novel tumour-associated antigens can be expected to significantly speed up the process of finding target molecules useful for tumour immunotherapy and diagnosis. Such identification of target molecules could be accomplished by the selection and screening of
15 antibody phage libraries on cultured tumour cells and tissue sections to generate specific reagents defining *in vitro* and *in vivo* expressed antigens (4). The phage display technology has been established as an efficient tool to generate monoclonal antibody reagents to various
20 purified antigens, and the construction and successful selection outcome from immune, naive and synthetic antibody phage libraries have been described in several studies (5).

25 Non-immune libraries are favourable with respect to their general applicability, making unique libraries for every single target unnecessary. On the other hand, sufficiently large and high quality non-immune libraries are difficult to construct and a target discovery process using these libraries should require efficient subtract-
30 ive selection methods when based on complex antigens.

 A phage library of a more moderate size has now been constructed from a near human primate immunised with complex human antigens. This represents an approach that takes advantage of an *in vivo* pre-selected repertoire.
35 Such libraries should be enriched for specificities to tumour specific epitopes in a reduced background reactivity to xenogeneic antigens (6). Furthermore, as

compared to the mouse, primate antibodies demonstrating close sequence homology with human antibodies should not be immunogenic in man (7).

Novel primate antibodies from a phage library that
5 define selectively expressed colon cancer associated
antigens have now been identified. The therapeutic
potential, demonstrated by T cell mediated killing of
cultured colon cancer cells coated with two of these
10 antibodies fused to engineered superantigens, is
comparable with superantigens fused to murine Fab
fragment specific for colon cancer associated antigens
such as EP-CAM, for which there has previously been
established the therapeutic capacity in experimental
systems (8).

15 There is also provided a method for efficient
positive and subtractive cell selection of phage
antibodies that should facilitate future identification
of novel phenotype specific antigens including tumour
associated antigens using antibodies from large phage
20 libraries.

BRIEF SUMMARY OF THE INVENTION

The present invention is related in a first aspect
to an antibody, or a derivative or a fragment thereof,
having a binding structure for a target structure
25 displayed in, and on the cell surface of, human
gastrointestinal epithelial tumour cells and in a
subpopulation of normal human gastrointestinal epithelial
cells, said binding structure comprising the complemen-
tarity determining region (CDR) sequences in the light
30 chain comprising essentially the amino acids number 23-33
(CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino acid
sequence shown in SEQ ID NO:2, and the CDR sequences in
the heavy chain comprising essentially the amino acids
number 158-162 (CDR1), 177-193 (CDR2), 226-238 (CDR3) of
35 the amino acid sequence shown in NO: 2, or other binding
structures with similar unique binding properties.

In one embodiment the antibody is phage selected. In another embodiment the sequences are of *Macaca fascicularis* origin. A further embodiment of the invention is a derivative of said antibody, which
5 derivative is of human origin. The sequences preferably have an identity of at least 84% to corresponding sequences of human origin. Preferably, the antibody has low immunogenicity or non-immunogenicity in humans.

In a further embodiment, the antibody has been
10 derivatised by genetically linking to other polypeptides, and/or by chemical conjugation to organic or non-organic chemical molecules, and/or by di-, oligo- or multimerisation.

In still a further embodiment, said antibody is
15 genetically linked or chemically conjugated to cytotoxic polypeptides or to cytotoxic organic or non-organic chemical molecules.

In a further embodiment, said antibody is
20 genetically linked or chemically conjugated to biologically active molecules.

In still a further embodiment, said antibody is genetically linked or chemically conjugated to immune activating molecules.

In another embodiment, said antibody has been
25 changed to increase or decrease the avidity and/or affinity thereof.

In still another embodiment, said antibody has been changed to increase the production yield thereof.

In a further embodiment, said antibody has been
30 changed to influence the pharmacokinetic properties thereof.

In still a further embodiment, said antibody has been changed to give new pharmacokinetic properties thereto.

35 In a further embodiment, said antibody is labeled and the binding thereof is inhibited by an unlabeled form of said antibody and not by other binding structures, and

not inhibiting the binding of other binding structures having other specificities.

A further embodiment is an antibody, the binding structure of which recognizes a non-reduced form of $\alpha 6\beta 4$ integrin.

In another aspect the invention relates to a target structure displayed in, or on the surface of, tumour cells, said target structure

a) having the ability of being specifically blocked by and to specifically block the binding structure of an antibody as defined in any one of claims 1-14, and other binding structures with similar binding specificities,

b) being displayed in, and on the surface of, human gastrointestinal epithelial cells,

c) having substantial homology with $\alpha 6$ and/or $\beta 4$ integrin chains or variants thereof, representing a shared or unique epitope,

d) being highly expressed on the surface of tumour cells, and

e) being a target for cytotoxic effector mechanisms.

By substantial homology in this context is meant homology in those parts of the target structure which are relevant for the binding of the antibody.

In one embodiment of said target structure, the binding structure is labeled and the binding thereof is inhibited by an unlabeled form of said binding structure and not by other binding structures, and not inhibiting the binding of other binding structures having other binding specificities.

In a further embodiment of said target structure said binding structure comprises one or more of the complementarity determining region (CDR) sequences comprising essentially the amino acids number 23-33, 49-55, 88-98, 158-162, 177-193, 226-238 of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties.

In still a further embodiment of said target structure said binding structure is an antibody, which antibody in a further embodiment comprises the variable region of a light chain comprising essentially the amino acids number 1-109 of the amino acid sequence shown in SEQ ID NO:2, and the variable region of a heavy chain comprising essentially the amino acids number 128-249 of the amino acid sequence shown in SEQ ID NO: 2.

Said target structure is in a further embodiment expressed homogenously in human colonic epithelial cells and less in pancreatic duct and bile duct cells.

In still a further embodiment, the expression of said target structure is correlated to gastrointestinal epithelial differentiation.

In another embodiment, said target structure comprises the amino acid sequence of $\alpha 6 \beta 4$ integrin, of which the $\alpha 6$ part is shown in SEQ ID NO: 3 and the $\beta 4$ part is shown in SEQ ID NO: 4. Another embodiment of the target structure comprises homo- or heteromonomers or homo- or heteromultimers of said $\alpha 6 \beta 4$ integrin and/or of said one or more fragments and/or variants and/or subunits thereof. Preferably, said target structure has an apparent molecular weight in its non-reduced form of from 90 to 140 kDa, most preferred from 80 to 160 kDa.

In still further embodiments the target structure comprises a peptide or polypeptide(s) comprising essentially any one of the amino acid sequences shown in SEQ ID NOS: 5-51, or comprises a molecule complexed to said polypeptide(s).

In the case of a target structure comprising amino acid sequences from the $\alpha 6 \beta 4$ integrin, said target structure may in a further embodiment be recognised, exclusively or not, in its non-reduced form by the binding structure comprised by the antibody as defined above.

The invention relates in a further aspect to a substance which binds to the target structure as defined

above, which substance is an organic chemical molecule or a peptide. In one embodiment, said substance is an anti-idiotypic of said target structure. Said anti-idiotypic may be specifically blocked by and specifically block a binding structure having similar binding specificity for said target structure.

In a still further aspect, the invention relates to a substance that blocks the function of the target structure as defined above, which substance is an organic molecule or a peptide.

In another aspect, the invention relates to a binding structure which recognises a target structure as defined above and which is of an organic chemical nature.

In a further aspect, the invention relates to a pharmaceutical composition comprising as an active principle an antibody as defined above, or a target structure as defined above, or a substance as defined above.

In still a further aspect, the invention is related to a vaccine composition comprising as an active principle an antibody as defined above, or a target structure as defined above, or a substance as defined above.

In a further aspect, the invention is related to a method of therapy for treating conditions based on an anti-angiotensin mechanism, whereby an antibody as defined above, or a target structure as defined above, or a substance as defined above, is administered to a human subject.

In another aspect, the invention is related to a method of treating human metastatic diseases, wherein an antibody as defined above is administered to a human subject.

In a further aspect the invention is related to a method of in vitro histopathological diagnosis and prognosis of human malignant disease, whereby a sample is

Another aspect of the invention is related to a method for therapy of human malignant disease, whereby an antibody, as defined above, is administered to a human subject. In one embodiment of this method said antibody has been changed by being genetically linked to molecules giving the combined molecule changed pharmacokinetic

properties. In another embodiment said antibody has been changed by being derivatised.

DETAILED DESCRIPTION OF THE INVENTION

The identification of novel tumour associated
5 antigens (TAAs) is pivotal for the progression in the
fields of tumour immunotherapy and diagnosis. In relation
to the present invention, there was first developed,
based on flow cytometric evaluation and use of a mini-
library composed of specific antibody clones linked to
10 different antibiotic resistance markers, methods for
positive and subtractive selection of phage antibodies
employing intact cells as the antigen source. An scFv
phage library (2.7×10^7) was constructed from a primate
(*Macaca fascicularis*) immunised with pooled human colon
15 carcinomas. This library was selected for three rounds by
binding to Colo205 colon adenocarcinoma cells, and
proteolytic elution followed by phage amplification.

Several antibodies reactive with colon carcinomas
and with restricted reactivity with a few epithelial
20 normal tissues were identified by immunohistochemistry.
One clone, A3 scFv, recognised an epitope that was
homogeneously expressed in 11/11 of colon and 4/4
pancreatic carcinomas studied and normal tissue
expression restricted to subtypes of epithelia in the
25 gastrointestinal tract. The A3 scFv had an apparent
overall affinity about 100-fold higher than an A3 Fab,
indicating binding of scFv homodimers. The cell surface
density of the A3 epitope, calculated on the basis of Fab
binding, was exceptionally high, approaching 3 million
30 per cell.

Efficient T cell mediated killing of colon cancer
cells coated with A3 scFv fused to the low MHC class II
binding superantigen mutant SEA(D227A) is also
demonstrated. The identified A3 molecule thus represents
35 a TAA with properties that suggests its use for immuno-
therapy of colon and pancreatic cancer.

DISCUSSION

In relation to the present invention, efficient protocols for phage selection to be used for the identification of cell phenotype specific antibody fragments from large phage libraries was developed. The target specificities for the applications as exemplified were for colon tumour associated antigens.

First the frequency of pIII-scFv fusion protein surface display in the phage population using the herein presented phagemid construct for phage propagation was analysed. A higher level of C215 scFv display was achieved as compared to previous reports. This should favour subtractive selection efficiency, but also increases the probability of avidity selection of low affinity antibodies from libraries.

Specificity of C215 scFv phage binding to colon adenocarcinoma Colo205 cells was clearly demonstrated. Bound phage could be efficiently eluted by use of the protease Genenase that specifically cleaves a target sequence between the phage protein III and the scFv antibody leaving the cells intact after elution. This non-chemical elution method should equally efficiently elute phage antibodies irrespectively of their binding affinity and only phage bound by scFv interactions, adding to the specificity of the process.

The enrichment achieved after three selection rounds on Colo205 cells (500 000x) using this selection protocol was similar to that reported by other investigators for selections on complex antigens.

After verifying the performance of the various methodological steps the combined technology was applied to library selections using Colo205 cells.

The library was constructed from a near human species immunised with human tumours. The antibody pool generated this way would potentially include affinity matured antibodies to tumour specific antigens in a limited background of xeno reactivities to widespread

normal human tissue antigens (6). The antibodies identified recognised tumour and tissue differentiation antigens with restricted normal tissue distribution. All of the selected antibodies identified as colon cancer tissue reactive in the primary screening also reacted with viable Colo205 cells in flow cytometry. This restriction to cell surface specificities should reflect the selection process and not the composition of the library, since a suspension of a mixture of tumour tissue components was used for the immunisation.

In a similar previous study extra- and intracellular specificities were identified in an anti-melanoma library produced the same way and selected using tissue sections as the antigen source (4). Tissue sections of resected human colorectal tumours and normal colon (mounted in the same well) were used for the primary screening using immunohistochemistry to assure the clinical relevance of the selected specificities, to increase the efficiency and to obtain more qualitative information as compared to flow cytometric screening.

The selected antibodies could be classified into four antibody specificity groups, distinguished by their reactivity patterns to epithelia in different organs (see Example 1, Table 1). Among these specificity groups, A3 scFv identified the most tumour selective antigen. This A3 TAA was highly, homogeneously and frequently expressed in samples of primary and metastatic colon cancer and of pancreatic cancer. Furthermore, its cell surface expression level as determined with the A3 Fab fusion protein (3 millions epitopes/cell) was exceptionally high and permissive for cell surface mediated cytotoxic effects.

Few, if any, of the frequently expressed human tumour antigens defined are tumour specific, but are commonly related to tissue differentiation such as A3 and the Ep-CAM. However, upregulated expression of these antigens in tumours should provide a basis for a

therapeutically active dose window. The availability from the circulation of normal tissue compartments expressing the antigen may also be more restricted due to limited capillary permeability and their site of expression in the body (e.g. the exposure of the apical side of gut epithelial cells to circulating antibodies should be very limited).

The clinical experience with the pan-epithelial Ep-CAM reactive 17-1A mAb supports the feasibility to identify an effective non-toxic antibody dose. The restricted expression in epithelia of all of the selected scFv clones in this work, indicate that these clones in principal could be evaluated as candidates for immunotherapeutic applications analogously to the 17-1A, e.g. as full-length mAbs. However, a particular advantage for the A3 TAA as compared to the Ep-CAM is the lack of expression in most normal epithelia such as of the lung and kidney, although the expression in the colon is similar.

The tissue distribution to subtypes of normal epithelia is supported by the selective expression in subtypes of carcinomas originating from the gastrointestinal tract (see Example 2, Table 2).

Several of the previously well-known colon cancer associated antigens (CEA, CA50, CA19-9, CA242, Tag-72) (3) are expressed equally or more restrictedly in normal tissues as compared to the A3 epitope. However, in contrast to the A3 and the C215 Ep-CAM they are more heterogeneously expressed in tumours.

Use of antibodies to the Ep-CAM has demonstrated good clinical results including a survival advantage for colorectal cancer patients in an adjuvant setting (2). With the objective to induce tumour responses even in more advanced stage patients, the introduction of potent effector molecules in conjunction with this antibody will challenge the "normal tissue resistance" seen in the treatment with the naked 17-1A mAb. Preclinically, this

could be studied in model systems using toxin-conjugated antibodies specific to the murine version of this antigen or animals transgenic for human colon cancer associated antigens.

5 Previously, antibody immunotoxins have been successfully used to cure mice in models with metastatically growing tumours expressing xeno (human) tumour antigens not expressed in mouse tissues (10). However, the TAAs used are truly tumour specific and the models do
10 not reflect the potential for normal tissue targeted toxicity.

In previous studies we have reported the potential of superantigens as immunostimulatory toxins for tumour immunotherapy (8). Antibody mediated targeting of
15 superantigens attracted large numbers of cytotoxic and cytokine-producing T cells to the tumour site. The superantigen SEA(D227A), mutated for low MHC class II binding affinity, was genetically linked to tumour targeting antibodies. This "tumour-selective" agent was
20 applied to recruit T cells independent of MHC expression in the tumour, thus short-cutting the problems of MHC down regulation and polymorphism that represent significant obstacles for other active immunotherapeutic approaches.

25 The mini-library of the established "tumour-selective", 1F scFv phage, the "broadly-reactive" C215 phage and the non-specific D1.3 phage antibody clones was an essential tool for the development of protocols for efficient subtractive cell selection. A requirement for
30 this selection principle is that the negative selection is followed by positive selection before phage rescue and amplification, due to the high frequency of non-displaying phage particles. Alternatively, non-displaying phage can be made non-infective by selective proteolysis
35 (G. Winter, pers. comm.). Such a technique may allow the generation of "inert libraries", i.e. libraries that have been extensively negatively preselected (e.g. towards a

cell in a resting state or a transfectable parental cell).

In conclusion, the "non-wanted" model phage specificity could selectively be subtracted from the phage population by a factor of approx. 100 for each selection round. Future subtractive selections using the developed protocol in combination with the use of large non-immune phage libraries for identification of differentially expressed cell surface antigens will demonstrate whether such an approach prove to be superior to the strategy we used in this study, i.e. positive selection using an in vivo pre-selected immune repertoire, including restrictions and biases such as immunodominance (4). The low affinity and high epitope density demonstrated for the A3 Fab binding to tumour cells as compared to the A3 scFv fusion protein suggests formation of scFv multimers that interact with epitopes that cluster on cell surfaces. Higher affinity monovalent variants of A3 Fab or alternatively, stable divalent constructs such as full-length A3 Fv grafted mAbs compatible with the putative low immunogenicity of A3 should be developed. Such constructs would be suitable for targeting of appropriate effector molecules to this highly expressed gastro-intestinal tumour associated antigen.

The invention is further illustrated in the following nonlimiting experimental part of the description.

EXMPERIMENTAL PART

Materials and Methods

30 Animals

Cynomolgus Macaque (*Macaca fascicularis*) monkeys were kept and immunised at the Swedish Institute for Infectious Decease Control (SIIDC), Stockholm. Water and food were always available *ad libitum*. Four monkeys were immunised subcutaneously with 2 ml of a crude suspension of colon cancer tissues in 10 % normal cynomolgus serum in PBS. Booster doses were given day 21, 35, and 49.

Antibody responses were demonstrated in two monkeys where the antigen had been admixed with alum adjuvant. All animals were kept according to Swedish legislation and the experiments were approved by the local ethical committees.

Tissues and cells

Human tumours and normal tissue samples were obtained from Lund University Hospital and Malmö General Hospital, Sweden. The human colorectal cell line Colo205, the human B cell lymphoma cell line Raji and the murine B16 melanoma cell line were from the American Tissue Culture Collection (ATCC, Rockville, MD). The mouse melanoma B16-C215⁺ cells transfected with the expression vector pKGE839 containing the Ep-CAM-1 gene (C215) has been described previously (9).

The human cells were cultured in RPMI 1640 medium (Gibco, Middlesex, UK) supplemented with 10% heat inactivated foetal bovine serum (Gibco) and 0.1 mg/ml gentamycin sulphate (Biological Industries, Kibbutz Beit Haemek, Israel). The mouse cells were cultured in medium additionally supplemented with 1 mM glutamine (Hyclone, Cramlington, UK), 5×10^{-5} M β -mercaptoethanol (ICN, Costa Mesa, CA), 0.2 % NaHCO₃ (Seromed Biochrome, Berlin, Germany), 1×10^{-2} M HEPES (HyClone, UT) and 1×10^{-3} M sodium pyrovate (HyClone). The cells were repeatedly tested for Mycoplasma contamination with Gene-Probe Mycoplasma T. C. test (San Diego, CA).

Phagemid vector and phage library construction

Total spleen RNA was extracted from one of the responding monkeys using an RNA isolation kit from Promega (Mannheim, Germany) and cDNA was amplified using an RNA PCR kit from PE Biosystems (Stockholm, Sweden). The primers for cDNA synthesis of lambda light chain and heavy chain genes and for the assembly of these genes to scFv genes have been reported previously (4). The scFv cDNA was ligated into a phagemid vector (4) in fusion with the residues 249-406 of the M13 gene III. The scFv-

gIII gene was expressed from a *phoA* promoter and the resulting protein was directed by the *E. coli* heat stable toxin II signal peptide.

Repeated electroporations of 7 μ g library vector
5 with scFv gene inserts resulted in a total of 2.7×10^7
primary transformed *E. coli* TG-1 growing as colonies on
minimal agar plates. The colonies were scraped from the
plates and grown in 2xYT at 150 rpm and 37°C for 1h. The
culture was superinfected with M13K07 helper phage
10 (Promega) in 50 times excess. Ampicillin to a concentra-
tion of 100 mg/l was added and the culture grown for a
further hour. After addition of kanamycin to a concentra-
tion of 70 mg/l, the culture was grown for 15 h at 30°C
and 250 rpm. The phage particles were harvested from the
15 culture supernatant using two repeated PEG/NaCl
precipitations. The precipitated phage was resolved in
PBS 1% BSA.

Western blot analysis

A two-fold dilution series of scFv-C215 phage
20 particles (from an undiluted stock of PEG-precip-
itated/concentrated phage) was applied to separation on a
reducing 12% polyacrylamide gel with 1% SDS and 2% β -
mercaptoethanol. The proteins were transferred to a
nitrocellulose membrane (Bio-Rad, Hercules, CA) by
25 electrophoresis. The membrane was blocked with 5% low-fat
milk (Semper AB, Stockholm, Sweden) and then incubated
with a rabbit antiserum against a protein III derived
peptide sequence, AEGDDPAKAAFNSLQASATEC, conjugated to
keyhole limpet hemocyanin. Secondary horse radish
30 peroxidase (HRP) conjugated goat-anti-rabbit antibodies
(Bio-Rad) were incubated for 30 min. Between all steps
the membrane was washed 3 times during 5 min in PBS/ 0.5%
Tween 20. The membrane was incubated in substrate
(Amersham Pharmacia Biotech, Little Chalfon Buckingham-
35 shire, UK) for one min. A light sensitive film (ECL
hyperfilm, Amersham) was exposed to the membrane and
developed for 0.5-5 min.

Similarly, to analyse the integrity of purified Fab (A3, including cynomolgus CH1 and Clambda domains), scFv- and Fab (including murine CH1 and Ckappa)-SEA(D227A) fusion proteins (produced as described previously (9)),

- 5 12% SDS-PAGEs were performed. The membranes with transferred proteins were incubated with purified polyclonal rabbit anti-SEA antibodies followed by the reagent steps described above.

Model and library phage selection on cells

- 10 Phage suspensions of the lambda light chain library (or of model phage), 10^{12} in 100 μ l PBS/1% BSA, were incubated with 3 million Colo205 cells for 1h on ice. The cells were washed 3 times including a 10-min incubation using 2 ml PBS/1% BSA for each wash. The phage were
15 eluted by adding 50 μ l of 33 μ g/ml Genenase to the cell pellet and incubated for 15 min. Genenase, which is a subtilisin BPN' mutant, S24C/H64A/E156S/G169A/Y217L, was kindly provided by Dr. Poul Carter (San Francisco, CA). After centrifugation the supernatant was transferred to a
20 new tube and 250 μ l 1% BSA in PBS was added. To rescue and amplify the selected library (and the model phage particles in the multi-pass experiment), the eluted phage particles were allowed to infect 1 ml, *E. coli* DH5 α F' (OD_{600 nm} = 1.0). The infected bacterial culture was
25 diluted 100 times with 2xYT supplemented with the proper antibiotic and cultured until an OD >1.0 (up to two days).

- Finally, to produce soluble scFv the amber suppressor strain HB2151 of *E. coli* was infected with the
30 selected library from the second and third round. After growth on agar plates containing ampicillin, single colonies were cultured in 96 Micro well plates in 2xYT medium supplemented with ampicillin at 30°C for 17 h. After centrifugation, removal of the supernatant to which
35 an equal volume of PBS/1%BSA was added, individual scFvs were analysed for immunoreactivity to sections of human tumours and normal tissues. Briefly, the C-terminal tag,

ATPAKSE, was detected using a rabbit antiserum followed by biotinylated goat anti-rabbit antibodies (DAKO A/S, Copenhagen, Denmark) and StreptABComplex HRP (DAKO A/S) (see "Immunohistochemistry").

5 *Immunohistochemistry*

Frozen cryosections (8 μ m) were air-dried on slides, fixed in acetone at -20°C for 10 min and rehydrated in 20% foetal bovine serum in PBS (FBS). Endogenous biotin was blocked with avidin (diluted 1/6) for 15 min and then
10 with biotin (diluted 1/6) for 15 min (Vector Laboratories, Burlingame, CA). Affinity purified and biotinylated rabbit anti-SEA antibodies, 5 μ g/ml, were incubated for 30 min followed by StreptABComplex HRP (DAKO A/S, Copenhagen, Denmark), 1/110 diluted in 50 mM
15 Tris pH 7.6 for 30 min. Between all steps the sections were washed 3 times in TBS. The staining reaction was developed for 8 min in 0.5 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (Sigma) dissolved in Tris pH 7.6 with 0.01 percent H₂O₂. After 10 min counterstaining in 0.5%
20 methyl green, the slides were rinsed for 10 min in tap water and gradually dehydrated in 70-99% ethanol and xylene before mounting in DPX medium (Sigma).

Flow cytometry

The Colo205 colon cancer cells were dissociated with
25 0.02% w/v EDTA and washed with PBS. To follow the development of an antibody response in the monkeys the cells were incubated consecutively with diluted serum, for 1h at 4°C, biotinylated rabbit anti-human IgG antibodies (Southern Biotechnology Ass. Inc., Al, USA)
30 for 30 min, and finally with avidin-PE (Becton Dickinson, Mountain View, CA) for 30 min.

The binding of model phage to the cells was analysed using rabbit-anti-M13 antibodies (produced by immunisation of rabbits with M13 particles) and FITC
35 conjugated donkey anti-rabbit antibodies (Amersham Pharmacia Biotech). The binding of antibodies fused to SEA(D227A) was detected using biotinylated rabbit anti-

SEA antibodies and avidin-PE. All reagents were diluted in PBS/1% BSA. The cells were washed twice with PBS/1% BSA after incubations with reagents and three times including 10 min incubations after binding of phage particles.

Flow cytometric analysis was performed using a FACSort flow cytometer (Becton Dickinson).

Affinity determination on cultured cells

A3 scFv-SEA(D227A), A3 Fab-SEA(D227A) and 1F scFv SEA(D227A) fusion proteins, 80 µg of each protein, were labelled with iodine as described by Bolton and Hunter to a specific activity of 10-15 µCi/µg. Colo205 cells and Raji cells, 30 000/sample were incubated with the iodinated fusion protein at 100 µl/tube in a two-fold dilution series in 1% BSA for 1h and then washed three times in PBS before measuring bound activity. The concentration of added and bound fusion protein was used for Scatchard analysis. The background binding to the Raji cells was subtracted to calculate the specific binding to the Colo205 cells.

Cytotoxicity assay

The T cell dependent cytotoxicity of the superantigen fusion protein (superantigen antibody dependent cellular cytotoxicity, SADCC) was measured in a standard 4 h chromium-release assay employing ⁵¹Cr-labelled Colo205 cells as target cells and human T cells as effector cells (9). The percent specific lysis was calculated as:

$$100 \times \frac{\text{cpm experimental release} - \text{cpm background release}}{\text{cpm total release} - \text{cpm background release}}$$

EXAMPLE 1

Generation of tumour binding monoclonal cynomolgus antibodies

Cynomolgus monkeys, *Macaca fascicularis* (four individuals) were repeatedly immunised with a suspension

of human colon carcinomas four times every other week. The gradual development of an antibody response in the monkeys was followed by flow cytometric staining of cultured colorectal cells, Colo205, using dilution series
5 of the preimmune and immune sera. An IgG antibody response was elicited only when alum precipitated tumour tissue suspensions were used (two individuals).

The monkey with the highest binding level of immune to preimmune serum antibodies was used for the construc-
10 tion of a large combinatorial scFv phage library of approximately 2.7×10^7 (estimated from the number of primary transformants). The primate phage library was selected using Colo205 cells. The total phage yield (eluted/added number of phage counted as colony forming
15 units, CFU) from three consecutive selection rounds increased gradually from 1.9×10^{-7} , 1.4×10^{-5} , to 1.2×10^{-3} . Five percent (12/246) of the monoclonal soluble scFv:s produced from the phage library after the third round of selection were demonstrated to bind to sections of a
20 human colorectal cancer tissue and to intact Colo205 cells by flow cytometry. All of the selected antibodies demonstrated individually unique nucleic acid sequences according to Hinf I restriction patterns analysed by 1% agarose gel electrophoresis.

25 The antibody genes were amplified by polymerase chain reaction using 5 μ l of bacterial cultures and primers complementary to regions 5' and 3' to the scFv gene in the phagemid vector (regions in the *phoA* promoter and in the M13 gene III).

30 *The selected scFv demonstrate individually unique reactivity with epithelia in normal tissues*

The colorectal cancer reactive scFv's were classified into specificity groups based on their immunohistochemical reactivity pattern with normal
35 tissues (Table 1). The antibodies studied in detail were A3 scFv (and A3 scFv-SEA(D227A)), A10 scFv, 3D scFv and 1D scFv. The representative antibodies could be

distinguished from each other by their fine specificity to epithelia in different organs and by their binding to leukocytes. The 1D scFv strongly reacted with gut epithelia and was the only antibody that reacted with
5 cells of polymorph nuclear granulocyte morphology. The 1D scFv also differed from the other antibodies by staining the luminal surface of kidney tubuli and collecting ducts whereas the A10 scFv reacted homogeneously (non-polarly) with these epithelial cells and 3D scFv and A3 scFv were
10 negative. 1D, A10 and 3D, but not A3 scFv also reacted with macrophage-like cells in the lung.

A fifth group of antibodies, not extensively evaluated and thus not included in Table 1, reacted with colon epithelia, leukocytes and Kupffer cells in the
15 liver. The A3 scFv stands out as demonstrating the most restricted reactivity with the panel of normal tissues used. The most prominent normal tissue reactivity of the A3 was staining of normal colon epithelium. Weak staining were also detected in small ducts of the pancreas and
20 bile ducts of the liver and of substructures in small bowel epithelia. The surface epithelium of one of the two stomach samples was strongly stained by the A3 antibody.

The reactivity pattern of the A3 scFv was confirmed using the fusion protein A3 scFv-SEA(D227A). This format
25 permitted the use of polyclonal rabbit anti-SEA antibodies for immunohistochemical detection, which is a more sensitive detection system demonstrating lower background and tissue crossreactivity as compared to the use of secondary antibodies to the peptide tag, ATPAKSE,
30 at the C-terminus of the scFvs.

Table 1 Immunohistochemical reactivity to normal human tissues of soluble scFv fragments from the selected colorectal cancer phage library
scFv clone designation

Tissue / sub-structure	n*	A3 **	A10	3D	1D
Esophagus / epithelial tissue	1	0	ND	ND	ND
/ non-epithelial tissue		0	ND	ND	ND
Colon / epithelium	5	++	+	+	++
/ non-epithelial tissue		0	0	0	granulocytes ++
Small bowel / villi epithelium	2	(+)	heterogenously	+	heterogenously (+)
/ basal glandulae		+	+	+	++
/ non-epithelial tissue		0	0	0	0
Ventricle / surface epithelium	2	0, ++	0	0, +	++
/ glandular epithelium		0	+, ++	0	++
/ non-epithelial tissue		0	0	0	0
Pancreas / acini	1	0	(+)	+	++
/ small ducts		(+)	(+)	+	++
/ large ducts		0	(+)	+	++
/ non-epithelial tissue		0	0	0	0
/ endocrine		0	0	0	0
Liver / hepatocytes	2	0	ND	ND	ND
/ Kupffer cells		0	ND	ND	ND
/ bile ducts		(+)	ND	ND	ND
Kidney / proximal tubuli	1	0	+	0	luminal surface ++
/ distal tubuli		0	+	0	luminal surface ++
/ collecting ducts		0	+	0	luminal surface ++
/ glomeruli		0	0	0	0
/ non-epithelial tissue		0	0	0	0
Bladder / epithelial tissue	1	0	ND	ND	ND
/ non-epithelial tissue		0	ND	ND	ND
Prostate / epithelial tissue	1	0	++	+	and secreted material ++
/ non-epithelial tissue		0	0	0	0
Lung / bronchial epithelium	1	0	(+)	(+)	0
/ alveolar epithelium		0	(+)	(+)	0
/ non-epithelial tissue		0	macrophages +	macrophages +	granulocytes ++, macrophages +
CNS / gray matter	1	0	ND	ND	ND
/ white matter		0	ND	ND	ND
Skeletal muscle	1	0	ND	ND	ND

Notes to Table 1

0 = negative, (+) = weak, + = moderate, ++ = strong, ND = not determined

* Number of tissue samples examined

- 5 ** The reactivity of A3 scFv has been confirmed with the A3 scFv SEA(D227A) fusion protein

EXAMPLE 2

10 The A3 tumour-associated antigen is homogeneously and frequently expressed in colorectal and pancreatic tumours

The A3 scFv-SEA(D227A) fusion protein was used for immunohistochemical staining of various tumours of epithelial origin (Table 2 and Figure 1). The fusion protein homogeneously and strongly stained 11/11 samples of primary colon cancer tissues and 4/4 metastatic colon cancer samples resected from the ovary, a lymph node and the liver. Pancreatic cancer tumours, 4/4 samples, were equally strongly positive. In contrast, tissue samples of gastric, prostate, breast and non-small cell lung carcinomas were negative.

20

Table 2 Tumor tissue reactivity of A3 scFv SEA(D227A)

Tumor tissue	n	Reactivity
Colon cancer, primary tumors	11	All tumor cells are strongly and homogenously stained
Colon cancer metastasis in lymph node, liver and ovary	4	As above
Pancreas cancer	4	As above
Ventricle cancer	2	Negative
Prostate cancer	2	Negative
Breast cancer	2	Negative
Lung carcinoma (non-small cell)	2	Negative
Malignant melanoma	2	Negative

EXAMPLE 3

The A3 TAA is highly expressed on the surface of colon cancer cells

The results from several Scatchard plots for
5 affinity determination, based on the binding of the
fusion proteins A3 scFv-SEA(D227A), A3 Fab and 1F scFv-
SEA(D227A) (1F was classified to the A3 specificity
group) to Colo205 cells, are summarised in Table 3.
Specific binding was calculated by subtraction of non-
10 specific binding to human B cell lymphoma Raji cells, a
cell line not expressing the A3 and 1F TAAs, from the
binding to Colo205 cells. Linear regression was used to
calculate the slope and the intercept of the extrapolated
line in the Scatchard plot. The A3 scFv-SEA(D227A) fusion
15 protein saturated approximately 10-fold less binding
sites per cell as compared to the A3 Fab (approx. 3
million sites per cell) fusion protein, indicating that
divalent (multivalent) binding was involved for the scFv.
This is supported by the more than 100-fold higher
20 overall affinity (3.6-5.5 nM) for the A3 scFv fusion
protein as compared to the A3 Fab (580-780 nM).

A single experiment performed using the 1F scFv-
SEA(D227A) fusion protein, demonstrated similar binding
affinity and saturation of binding sites as the A3 scFv-
25 SEA(D227A) fusion protein.

Table 3 Scatchard analysis of iodinated fusion
proteins binding to Colo205 cells

Fusion protein	n*	Kd (nM)	million sites /cell
A3 Fab-SEA(D227A)	2	580-780	3.0-3.9
A3 scFv-SEA(D227A)	3	3.6-5.5	0.11-0.39
1F scFv-SEA(D227A)	1	4.2	0.18

* Number of experiments performed

EXAMPLE 4

A3 and 1F scFv-SEA(D227A) mediate T cell lysis of Colo205 cells

The capacity of the two fusion proteins A3 and
 5 1F scFv-SEA(D227A) to mediate superantigen antibody
 dependent cellular cytotoxicity (SADCC) towards Colo205
 cells was investigated and compared with the positive
 control C215 Fab-SEA(D227A) and negative control
 D1.3 scFv-SEA(D227A) fusion proteins. The A3 scFv-
 10 SEA(D227A) fusion protein titration approached a plateau
 for maximal lysis which was similar, approx. 50 percent
 in this 4 h assay, to that demonstrated for the C215 Fab-
 SEA(D227A) fusion protein, although at a ten-fold higher
 concentration (Figure 2). The 1F scFv-SEA(D227A) mediated
 15 a similar level of cytotoxicity at a slightly higher
 concentration as compared to the A3 scFv-SEA(D227A).

The negative control D1.3 scFv SEA(D227A) fusion
 protein did not mediate any cytotoxicity.

EXAMPLE 5

20 *Purification of a tumour associated antigen that is
 recognised by the colon cancer reactive antibody A3.*

A tumour extract was made out of xenografted tumour
 cell line Colo205. The extract was applied to a pre-
 column coupled with C215Fab-SEAm9, and a column coupled
 25 with A3scFv-SEAm9. The columns were in series, during
 the application of sample but separated prior to elution
 under alkaline conditions.

A single peak was detected during elution by UV
 spectroscopy (Figure 3). This eluted fraction from the
 30 latter A3-column was collected, neutralised,
 concentrated, and then analysed by SDS-PAGE under non-
 reducing conditions (Figure 4). Two bands visible by
 silver staining (labelled I and II in Figure 4) of
 apparent molecular weight of approximately 90-140 kDa
 35 were cut out and examined by standard peptid mapping
 methodologies. These two bands corresponds to bands
 detected by A3 in Western Blot, see example 8. From band

- I 47 separate tryptic peptide masses were obtained (see SEQ ID NO: 3, Table 4, and Fig 5 for the sequences and corresponding mass weights) which completely matched to different tryptic peptide masses. as determined by MALDI-TOF) of the human $\alpha 6$ integrin or $\beta 4$ integrin (see SEQ ID NOs: 5-51 and 3-4, respectively, and Fig 3A and B, respectively, where in Fig 3A the underlinings correspond to the peptides appearing in Fig 3B/SEQ ID NOs: 5-51). From band II 22 separate tryptic peptide masses were obtained which completely matched to different tryptic peptide masses of $\beta 4$ integrin (data not shown). The data show that the $\alpha 6\beta 4$ integrin heterodimer is specifically isolated with the A3-affinity column.
- Table 4 *Peptides/polypeptides derived from human $\alpha 6\beta 4$ integrin and masses thereof*

Sequence No.	Sequence	Measured Mass	Calculated Mass
5	LLLVGAPR	838.568	838.551
6	ANRTGGLYSCDITARGPCTR	2226.131	2226.050
7	VVTCAHRYEK	1262.637	1262.631
8	RQHVNTK	882.524	882.490
9	CYVLSQNLK	1152.618	1152.583
10	FGSCQQGVAATFTK	1501.706	1501.710
11	DFHYIVFGAPGTYNWK	1914.881	1914.917
12	DEITFVSGAPR	1191.625	1191.600
13	ANHSGAVVLLK	1108.600	1108.647
14	DGWQDIVIGAPQYFDR	1879.865	1879.897
15	DGEVGGAVYVYMNQOGR	1842.811	1842.844
16	WNNVKPIR	1026.608	1026.584
17	NIGDINQDGYPDIAVGAPYDDLK	2520.213	2520.189
18	GISPYFGYSIAGNMDLDR	1975.913	1975.922
19	NSYPDVAVGSLSDSVTIFR	2026.992	2027.008
20	SRPVINIQK	1054.644	1054.637
21	LRPIITASVEIQEPSSR	1993.066	1993.108
22	VNSLPEVLPIILNSDEPK	1863.920	1864.006
23	TAHIDVHFLK	1180.665	1180.647
24	FSYLPQK	995.601	995.556
25	DIALEITVTNSPSNPR	1726.866	1726.897
26	SEDEVGSLIEYEFK	1672.764	1672.770
27	VESKGLEKVTCEPQK	1731.866	1731.895

28	REITEKQIDDNRK	1644.792	1644.866
29	FSLFAER	869.476	869.452
30	YQTLNCSVNVNVCVNR	1954.003	1953.927
31	LNLYDILMR	1150.644	1150.629
32	AFIDVTAAAENIR	1390.739	1390.733
33	LPNAGTQVR	955.523	955.532
34	VSVPTQDMRPEK	1386.727	1386.705
35	EPWPNSDPPFSFK	1547.730	1547.717
36	NVISLTEDVDEFR	1536.744	1536.754
37	TQDYPSVPTLVR	1375.718	1375.722
38	RGEVGIYQVQLR	1417.801	1417.791
39	ALEHVDGTHVCQLPEDQK	2075.965	2075.981
40	GNIHLKPSFSDGLK	1512.749	1512.817
41	MDAGIICDVCTCELOK	1928.901	1928.822
42	YEGQFCEYDNFQCPR	2012.795	2012.790
43	SCVQCQAWGTGEKKGR	1879.865	1879.890
44	DEDDDDCTYSYTMEGDGAPGNSTVL VHK	3103.229	3103.278
45	QEVEENLNEVYR	1521.779	1521.718
46	VAPGYTTLTADQDAR	1640.779	1640.791
47	VPLFIRPEDDDEK	1572.778	1572.790
48	DVVSFEQPEFSVSR	1625.758	1625.781
49	LLELQEVDSSLR	1427.760	1427.810
50	VCAYGAGQEGPYSSLVSCR	2060.883	2060.916
51	VLVDNPKNR	1054.644	1054.600

Materials and Methods.

Solubilisation of Tumour Tissue

- 5 Human colon cancer tissue expressing the A3 antigen was provided by hospitals in Sweden and stored frozen at -70°C in the tissue bank at ABR. Frozen colon cancer tissues were sliced with a scalpel and transferred into a tube containing cold isotonic sucrose buffer (0.25M
- 10 sucrose, 10mM KCl, 1.5M MgCl₂, 50mM Tris-HCl pH 7.4 at 25°C) containing 1% (v/v) Nonidet P-40 (NP-40) and protease inhibitors (CompleatTM Protease Inhibitor Cocktail Tablet, Boehringer Mannheim). Tissue was homogenised with an Ultra-Turrax homogeniser and were
- 15 left to solubilise at 0°C. The solubilised preparation was centrifuged at 11,000 rpm (Hettich centrifuge Universal 30 RF rotor), to remove cell debris. The supernatant was further centrifuged at 108,000g at 4°C

(Beckman Ultracentrifuge Ti-60 rotor), and finally filtered through a 0.2 μ m Minisart plus filter (Sartorius AG Gottingen Germany).

Affinity Purification of tissue antigens

5 A3scFv-SEAm9 was coupled to a NHS-activated HiTrap[®] column (Pharmacia Biotech Uppsala Sweden), according to the manufacturer's recommendations. The control and pre-column were coupled with C215Fab-SEAm9, and the control, pre-column and column were set up in series. All columns
10 were washed with pre-wash buffer (20mM Tris HCl pH7.5 at 4°C containing 0.2% NP 40). The extract was loaded onto the column at 0.1ml/min, and the flow through was recirculated. The columns were then washed with start buffer. Bound antigen was eluted in a pH gradient of
15 diethylamine starting at pH 7.5 up to 11.0. 2.5 ml of eluant was collected and concentrated down to 75 μ l. The purification was performed at 4°C using an AKTA FPLC system (Amersham Pharmacia Biotech Uppsala Sweden). Eluted protein was analysed by SDS PAGE and silver
20 staining. Individual bands were excised, digested with trypsin and the masses of the peptide were determined using a MALDI-TOF instrument by Protana A/S (Odense, Denmark). The peptide masses were then compared in a computer search with all tryptic peptide masses for each
25 protein in the SWISSPROT database, a service provided by Protana A/S (Odense Denmark).

EXAMPLE 6

A3scFv-SEAm9 detects a novel α 6 β 4 integrin epitope

30 Commercial antibodies to human α 6 integrin and β 4 integrin were compared to A3 on normal and malignant colon sections. The reactivity, shown in Figure 6, demonstrates that A3 is restricted to the colon epithelium (Fig 6[i]), and malignant cell in the tumour (Fig 6 [ii]). Commercial antibody NKI-GoH3 to α 6
35 integrin, also reacted with normal colon (Fig 6 [iii]) and colon cancer (Figure 6 [iv]). Reaction was seen in epithelial cells of colon and malignant cells (arrows)

but also in blood vessels (BV), some stromal components (s) and in muscularis mucosae (mm). The reaction observed with commercial ASC-3 anti- β 4 integrin antibody was similar to that noted with anti- α 6 antibody but weaker, in both normal colon (v) and colon cancer (vi).

Materials and Methods

Antibody

A3 scFv was selected from the M fascicularis library. The VH and VL genes from this were released by restriction enzyme digestion and fused to the Staphylococcal Enterotoxin AE chimeric mutant (D227A) to generate the A3scFv-SEAm9. This demonstrated very low levels of non-specific binding and allowed sensitive detection by secondary antibodies. ASC-3 anti-human- β 4 integrin antibody and NKI-GoH3 anti-human- α 6 integrin antibody were from Becton Dickinson (Copenhagen, Denmark)

Immunohistochemistry

Tumour and normal tissue samples were obtained from the Department of Surgery Lund Hospital. These were rate-frozen in iso-pentane, which had been pre-cooled in liquid nitrogen. Samples were stored at -70°C until sectioned. After cryosectioning the sections were air dried over night, fixed in cold acetone and blocked with avidin/biotin (Vector Burlingame CA). Primary antibody was then added to the section for one hour.

The secondary antibodies were incubated for 30 minutes followed by streptavidin-biotin/HRP (Dakopatts Copenhagen Denmark) for a further 30 minutes. Extensive washing was performed between all these steps with 50mM Tris pH 7.6, 0.15M NaCl. Diaminobenzidine (DAB) was used as chromogen and the sections were counterstained in 0.5% methyl green. Controls included a non-tissue reactive Fab and SEA-D227A or no primary antibody. All antibodies were used at a final concentration of 5 $\mu\text{g}/\text{ml}$. Results were expressed as negative, weak, moderate or strong staining.

EXAMPLE 7

The A3 Tumour Associated Antigen reacted with $\alpha 6$ and $\beta 4$ integrin antibodies in a capture ELISA

Crude tumor extract or A3 antigen purified by A3-
5 affinity chromatography (see example 5) was analysed by a
capture ELISA. Commercial antibody ASC-3 specific for
beta 4 integrin were used as capture antibody, to which
different dilutions of crude tumor extract was applied.
This was then chased with A3scFv-SEAm9. Bound A3scFv-
10 SEAm9 was then detected with anti-SEA-HRP (Fig 7A). In
Figure 7B the commercial anti- $\alpha 6$ integrin antibody NKI-
GoH3 was used to capture different dilutions of the
concentrated A3-affinity purified eluate. In a similar
way as in Figure 7A the captured proteins were chased
15 with A3scFv-SEAm9 and detected with anti-SEA-HRP. In both
experiments a concentration dependent signal was
detected. These results confirm the specificity of A3 to
 $\alpha 6 \beta 4$ integrin heterodimer, which was also shown to be
specifically isolated from the A3-affinity column in
20 example 5.

Material and Methods

Commercial antibodies NKI-GoH3 or ASC-3 (Becton
Dickinson Copenhagen Denmark) 100 μ l, were used to coat
the well of an E.I.A./R.I.A.-plate (Costar) in 0.05 M
25 NaHCO₃, pH 9.6. The reaction was allowed to continue
overnight at 4°C, after which the plates were washed 4
times in DPBS + 0.05 % Tween 20. Wells were then blocked
with 200 μ l 3 % non-fat milk powder in DPBS + 0.05 %
Tween 20, for 1-2 h at room temperature (RT) with
30 shaking. Wells were again washed as above and 100 μ l
antigen extract diluted in 3 % non-fat milk powder in
DPBS + 0.05 % Tween 20, was applied for 2 h at RT with
shaking. Wells were again washed (4 x DPBS + 0.05 % Tween
20) after which 100 μ l of the primary antibody diluted in
35 3 % non-fat milk powder in DPBS + 0.05 % Tween 20 was
incubated for 2 h at RT with shaking. Wells were washed
again as above and 100 μ l of the secondary antibody

diluted in 3 % non-fat milkpowder in DPBS + 0.05 % Tween 20 was added to each well for 1 h at RT with shaking. Wells were again washed as above and colour developed by the addition of 100 μ l peroxidase substrate (Sigma Fast OPD Peroxidase Substrate Tablet Set P-9187). The reaction was allowed to continue for 30 min at RT, in the dark and shaking before the reaction was stopped by the addition of 50 μ l 3 M H_2SO_4 . The absorbance was read at 490 nm.

10 EXAMPLE 8

Western Blot analysis of the A3 tumour antigen

A3-affinity purified tumour antigen extracts were separated by SDS-PAGE and transferred to membranes for Western blot analysis. Extracts were applied directly or heated to 100°C for 5 minutes or heated to 100°C for 5 minutes but in the presence of mercaptoethanol (BME) (Figure 8). The membranes were then probed with A3scFv-SEAm9 and anti-SEA-HRP or anti-human- $\alpha 6$ integrin or anti-human- $\beta 4$ integrin antibodies. The anti- $\beta 4$ integrin antibody did not react with any protein on the membrane (Fig 8[ii]). The anti-human- $\alpha 6$ integrin reacted with a major specie with apparent molecular weight between 90 - 140 kDa in the A3-affinity purified tumour antigen extract (Figure 8[iii]). The same species was also detected by A3scFv-SEAm9, which also was detected after heating but was much weaker under reduced conditions (with BME present) (Figure 8[i]). The major band detected in the 90 - 140 kDa interval corresponds to the bands in example 5, that were analysed by peptide mapping and were found to contain $\alpha 6$ integrin and $\beta 4$ integrin.

Materials and Methods

ASC-3 anti-human- $\beta 4$ integrin antibody and NKI-GoH3 anti-human- $\alpha 6$ integrin antibody were from Becton Dickinson (Copenhagen, Denmark). Samples were resolved by SDS-PAGE in 0.25M tris-glycine pH 8.9 and 0.1%SDS at 100V through the upper gel, then 170V through the resolving gel.

Molecular weight standards (Biorad broad Range, Biorad) were included on all gels. Resolved samples were transferred to nitrocellulose (Biorad) in transfer buffer (10 mM Tris base, 2M glycine, 40% (v/v) methanol) at 100V
5 for 1 hour. Membranes were blocked with 5% (w/v) BSA/TBS for at least 2 hours at 4°C, then incubated with the appropriate antibody diluted in 5% BSA/TBS/0.2% azide. This reaction was allowed to proceed for at least 2 hours at RT, after which the membrane was washed extensively in
10 TBST-T. Bound antibody was detected by incubation of membranes for 1 hour with HRP conjugated antibody diluted in TSB-T containing 5% milk powder. Membranes were then incubated with enhanced chemiluminescence (ECL) detection reagents (Renaissance® NEN™ Life Science Products, Boston
15 MA) for 1 minute and exposed to film for up to 1 hour.

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LEGENDS TO FIGURES

Figure 1 The A3 tumour-associated antigen is homogeneously expressed in primary and metastatic tumours

Immunohistochemical staining of frozen and acetone
5 fixed sections of human tumour tissues using A3 scFv-SEA(D227A) and C215 Fab-SEA(D227A) at 70 nM. The A3 scFv fusion protein reacted strongly and homogeneously with both primary colon and pancreatic carcinoma resected from tumour patients. A representative staining of a primary
10 colon cancer is shown for C215 Fab-SEA(D227A) in (A) and for A3 scFv-SEA(D227A) in (B). Staining by A3 scFv-SEA(D227A) of a colon cancer liver metastasis is shown in (C) and of a primary pancreatic cancer in (D).

Figure 2 A3 scFv-SEA(D227A) coated Colo205 tumour cells
15 are efficiently killed by T cells.

Superantigen antibody dependent cellular cytotoxicity (SADCC) towards Colo205 cells mediated by A3 scFv-SEA(D227A) reached the same maximal cytotoxicity as the anti-Ep-CAM fusion protein C215 Fab-SEA(D227A),
20 although at a ten-fold higher concentration. The absence of cytotoxicity mediated by the D1.3 scFv-SEA(D227A) demonstrates the need of a tumour targeting antibody moiety in the fusion protein.

Figure 3

25 Immunoaffinity chromatography of tumor extract on a A3scFv-SEAm9 coupled column. Protein bound to A3 coupled columns was washed extensively then eluted as described in Materials and Methods in Example 5. The eluted fractions were examined by UV spectroscopy (arrow) and a
30 single peak identified. The sample was eluted with a pH gradient as indicated by an x.

Figure 4

A3 antigen preparation was separated on a non-reduced SDS PAGE and silver-stained. Previous Western
35 analysis had defined a molecular weight range in which the A3 antigen was believed to reside. The bands evident

within this region (Labelled I and II) were excised for peptide mapping analysis

Figure 5A and 5B

5 Epithelial integrin $\alpha 6 \beta 4$: complete primary structure of $\alpha 6$ and variant forms of $\beta 4$ (precursor) (Tamura et al J Cell Biol 111:1593-1604 (1990)). The matched peptides shown in SEQ ID NOs: 5-51 are underlined in the sequences of human $\alpha 6$ (Fig 5A) integrin and $\beta 4$ (precursor) (Fig 5B) integrin as published.

10 Figure 6

Immunohistochemistry of normal and malignant colon using A3scFv and commercial anti-human $\alpha 6$ and $\beta 4$ integrin monoclonal antibodies.

Figure 7A and 7B

15 Capture ELISA. In fig 7A monoclonal antibody ASC-3 specific for $\beta 4$ integrin was used as capture antibody, to which different dilutions of crude tumor extract was applied. In fig 7B the anti- $\alpha 6$ integrin monoclonal antibody NKI-GoH3 was used to capture different dilutions of
20 the concentrated A3-affinity purified eluate. In both fig 7A and 7B the captured integrin antigen was then successfully detected with A3scFv-SEAm9.

Figure 8A and 8B

25 Western blot analysis of the eluate from the A3-affinity column. The primary antibodies used are (i) and (ii) A3scFv-SEAm9, (iii) ASC-3 anti-human- $\beta 4$ integrin antibody and (iv) NKI-GoH3 anti-human- $\alpha 6$ integrin antibody. Lane A - the eluate was applied directly, lane B - the eluate was heated to 100°C for 5 minutes, and
30 lane C - the eluate was heated to 100°C for 5 minutes but in the presence of mercaptoethanol. Positions of molecular weight standards are indicated.

CLAIMS

1. An antibody, or a derivative or a fragment
5 thereof, having a binding structure for a target
structure displayed in, and on the cell surface of, human
gastrointestinal epithelial tumour cells and in a
subpopulation of normal human gastrointestinal epithelial
cells, said binding structure comprising the
10 complementarity determining region (CDR) sequences in the
light chain comprising essentially the amino acids number
23-33 (CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino
acid sequence shown in SEQ ID NO:2, and the CDR sequences
in the heavy chain comprising essentially the amino acids
15 number 158-162 (CDR1), 177-193 (CDR2), 226-238 (CDR3) of
the amino acid sequence shown in SEQ ID NO:2, or other
binding structures with similar unique binding
properties.

2. An antibody according to claim 1, which is phage
20 selected.

3. An antibody according to claim 1, wherein the
sequences are of *Macaca fascicularis* origin.

4. A derivative of an antibody according to claim 1,
which is of human origin.

25 5. An antibody according to claim 1, wherein the
sequences have an identity of at least 84% to correspond-
ing sequences of human origin.

6. An antibody according to claim 1, which has low
immunogenicity or non-immunogenicity in humans.

30 7. An antibody according to claim 1, which has been
derivatised by genetically linking to other polypeptides,
and/or by chemical conjugation to organic or non-organic
chemical molecules, and/or by di-, oligo- or
multimerisation.

35 8. An antibody according to claim 1, which is
genetically linked or chemically conjugated to cytotoxic

polypeptides or to cytotoxic organic or non-organic chemical molecules.

9. An antibody according to claim 1, which is genetically linked or chemically conjugated to biologically active molecules.

10. An antibody according to claim 1, which is genetically linked or chemically conjugated to immune activating molecules.

11. An antibody according to claim 1, which has been changed to increase or decrease the avidity and/or affinity thereof.

12. An antibody according to claim 1, which has been changed to increase the production yield thereof.

13. An antibody according to claim 1, which has been changed to influence the pharmacokinetic properties thereof.

14. An antibody according to claim 1, which has been changed to give new pharmacokinetic properties thereto.

15. An antibody according to claim 1, which is labeled and the binding thereof is inhibited by an unlabeled form of said antibody and not by other binding structures, and not inhibiting the binding of other binding structures having other binding specificities.

16. An antibody according to claim 1, wherein said binding structure recognises a non-reduced form of $\alpha 6\beta 4$ integrin.

17. A target structure displayed in, or on the surface of, tumour cells, said target structure

a) having the ability of being specifically blocked by and to specifically block the binding structure of an antibody as defined in any one of claims 1-14, and other binding structures with similar binding properties,

b) being displayed in, and on the surface of, human gastrointestinal epithelial cells,

c) having substantial homology with $\alpha 6$ and/or $\beta 4$ integrin chains or variants thereof, representing a shared or unique epitope,

d) being highly expressed on the surface of tumour cells, and

e) being a target for cytotoxic effector mechanisms.

18. A target structure according to claim 17,
5 wherein the binding structure is labeled and the binding thereof is inhibited by an unlabeled form of said binding structure and not by other binding structures, and not inhibiting the binding of other binding structures having other binding specificities.

10 19. A target structure according to claim 17, wherein said binding structure comprises one or more of the complementarity determining region (CDR) sequences comprising essentially the amino acids number 23-33,
15 49-55, 88-98, 158-162, 177-193, 226-238 of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties.

20. A target structure according to claim 17, wherein said binding structure is an antibody.

21. A target structure according to claim 20,
20 wherein said antibody comprises the variable region of a light chain comprising essentially the amino acids number 1-109 of the amino acid sequence shown in SEQ ID NO:2, and the variable region of a heavy chain comprising essentially the amino acids number 128-249 of the amino
25 acid sequence shown in SEQ ID NO: 2.

22. A target structure according to any one of claims 17-21, which is expressed homogenously in human colonic epithelial cells and less in pancreatic duct and bile duct cells.

30 23. A target structure according to any one of claims 17-22, the expression of which is correlated to gastrointestinal epithelial differentiation.

24. A target structure according to any one of claims 17-23, which comprises essentially the amino acid
35 sequence of $\alpha 6$ integrin shown in SEQ ID NO: 3 and/or of $\beta 4$ integrin shown in SEQ ID NO: 4, and/or one or more

fragments, and/or variants or splice variants, and or subunits, thereof.

25. A target structure according to claim 24, which comprises homo- or hetero-monomers or homo- or hetero-
5 multimers of said $\alpha 6\beta 4$ integrin and/or of said one or more fragments and/or variants and/or subunits thereof.

26. A target structure according to claim 24, which has an apparent molecular weight in its non-reduced form of from 90 to 140 kDa, most preferred from 80 to 160 kDa.

10 27. A target structure according to claim 24, which comprises a peptide or polypeptide(s) comprising essentially any one of the amino acid sequences shown in SEQ ID NOs: 5-51, or comprises a molecule complexed to said polypeptide(s).

15 28. A target structure according to any one of claims 24-27 recognised, exclusively or not, in its non-reduced form by the binding structure comprised by the antibody as defined in any one of claims 1-16.

20 29. A substance which binds to the target structure as defined in any one of claims 17-28, which substance is an organic chemical molecule or a peptide.

30. A substance, which is an anti-idiotypic of a binding structure to said target structure as defined in anyone of claims 17-28.

25 31. A substance according to claim 30, which anti-idiotypic is specifically blocked by and specifically blocks a binding structure having binding specificity for said target structure.

30 32. A substance which blocks the function of the target structure as defined in any one of claims 17-28, which substance is an organic chemical molecule or a peptide.

35 33. A binding structure which recognizes a target structure as defined in any one of claims 17-28, and which is of an organic chemical nature.

34. A pharmaceutical composition comprising as an active principle an antibody as defined in any one of claims 1-16.

5 35. A pharmaceutical composition comprising as an active principle a target structure as defined in any one of claims 17-28.

36. A pharmaceutical composition comprising as an active principle a substance as defined in any one of claims 29-32.

10 37. A vaccine composition comprising as an active principle an antibody as defined in any one of claims 1-16, or a target structure as defined in any one of claims 17-28, or a substance as defined in any one of claims 29-32.

15 38. A method of therapy for treating conditions based on an anti-angiogenic mechanism, whereby an antibody as defined in any one of claims 1-16, or a target structure as defined in any one of claims 17-28, or a substance as defined in any one of claims 29-32, is
20 administered to a human subject.

39. A method of treating human metastatic diseases, wherein an antibody as defined in any one of claims 1-16 is administered to a human subject.

25 40. A method of in vitro histopathological diagnosis and prognosis of human malignant disease, whereby a sample is contacted with an antibody as defined in any one of claims 1-17 and an indicator.

41. A method according to claim 40, which method comprises tumour typing.

30 42. A method according to claim 40, which method comprises tumour screening.

43. A method according to claim 40, which method comprises tumour diagnosis and prognosis.

35 44. A method according to claim 40, which method comprises monitoring premalignant conditions.

45. A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily

fluids of an antigen comprising a target structure, as defined in any one of claims 17-28,

46. A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of an antibody as defined in any one of claims 1-16 is assayed.

47. A method for in vitro diagnosis and prognosis of human malignant disease, whereby concentrations in bodily fluids of a complex of a) an antigen comprising a target structure, as defined in any one of claims 17-28, or a structure, as defined in any one of claims 29-32, is assayed, and b) an antibody, as defined in any one of claims 1-16, is assayed.

48. A method for in vivo diagnosis and prognosis of human malignant disease, whereby the localisation of an antibody, as defined in any one of claims 1-16, to tumour deposits in a human subject is determined.

49. A method according to claim 48, whereby said antibody is administered to the subject before the determination.

50. A method according to claim 48, whereby said antibody is accumulated in tumour deposits.

51. A method according to any one of claims 48-50, which is quantitative.

52. A method for therapy of human malignant disease, whereby an antibody, as defined in any one of claims 1-16, is administered to a human subject.

53. A method according to claim 52, whereby said antibody has been changed by being genetically linked to molecules giving the combined molecule changed pharmacokinetic properties.

54. A method according to claim 52, whereby said antibody has been changed by being derivatised.

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9903895-2 28 October 1999 (28.10.1999) SE(71) Applicant (for all designated States except US): **ACTIVE BIOTECH AB** [SE/SE]; Box 724, S-220 07 Lund (SE).

(72) Inventors; and

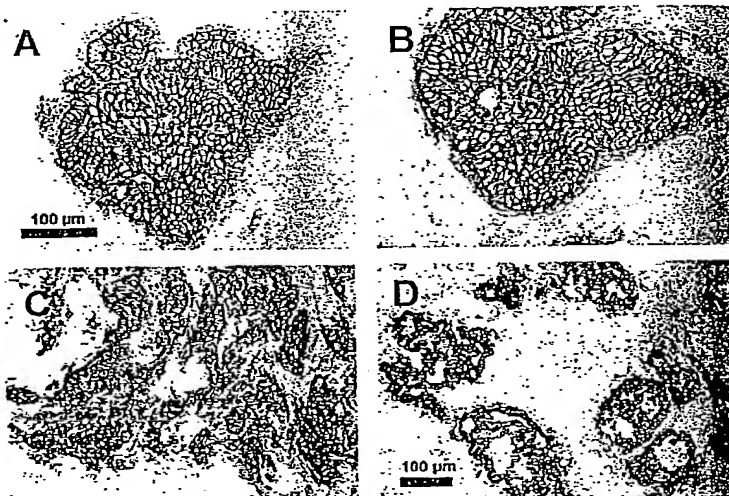
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(81) Designated States (national): AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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[Continued on next page]

(54) Title: NOVEL COMPOUNDS



(57) Abstract: An antibody, or a derivate or a fragment thereof, having a binding structure for a target structure is described. The antibody is displayed in, and on the cell surface of, human gastrointestinal epithelial tumour cells and in a subpopulation of normal human gastrointestinal epithelial cells. Said binding structure comprises the complementarity determining region (CDR) sequences in the light chain comprising essentially the amino acids number 23-33 (CDR1), 49-55 (CDR2), 88-98 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, and the CDR sequences in the heavy chain comprising essentially the amino acids number 158-162 (CDR1), 177-193 (CDR2), 226-238 (CDR3) of the amino acid sequence shown in SEQ ID NO:2, or other binding structures with similar unique binding properties. There is also described a target structure displayed in, or on the surface of tumour cells, vaccine compositions, pharmaceutical compositions as well as methods related to human malignant diseases.

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— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

FIG. 1

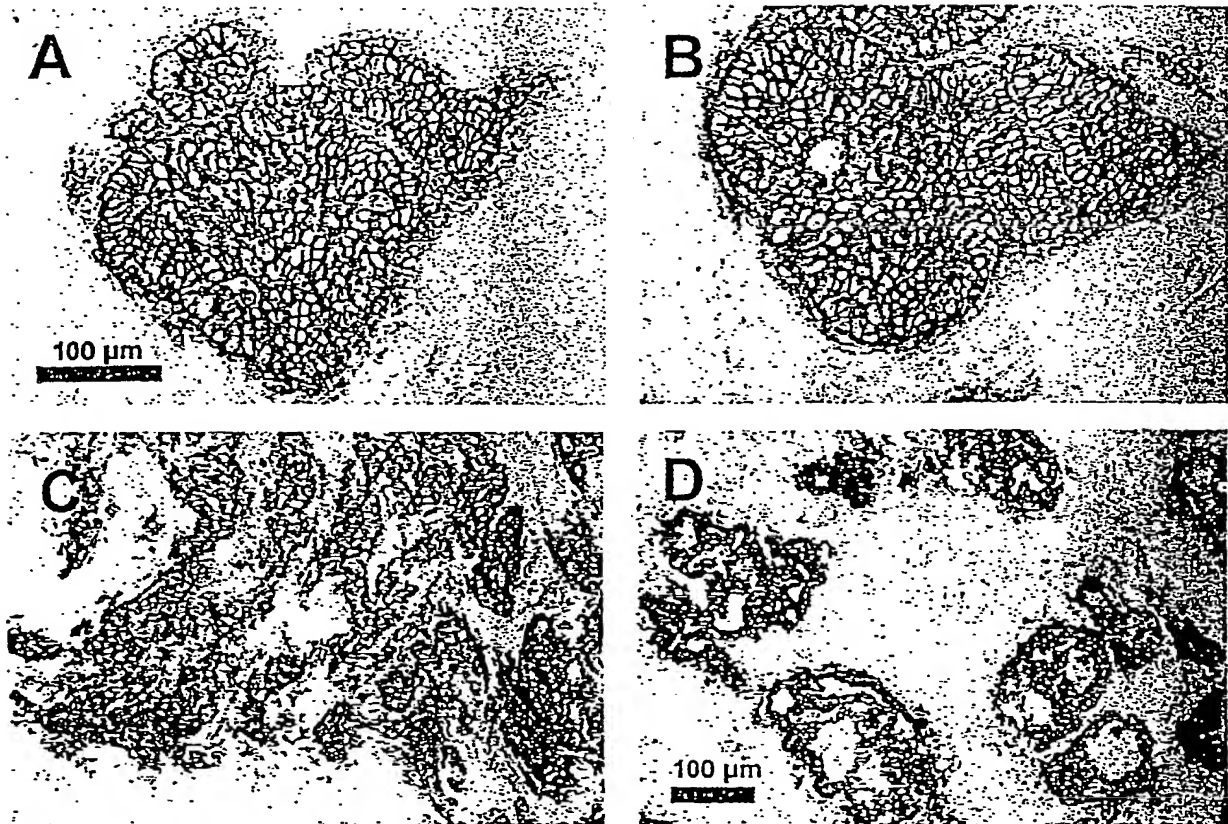
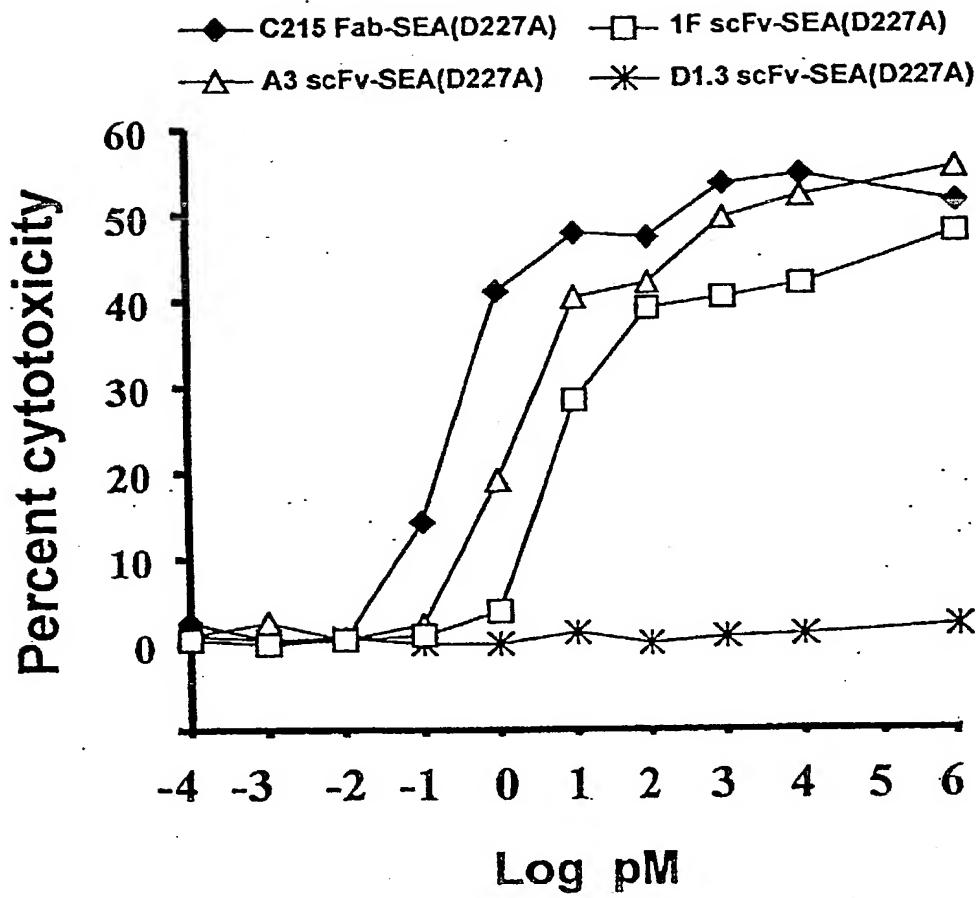


FIG. 2



3/10

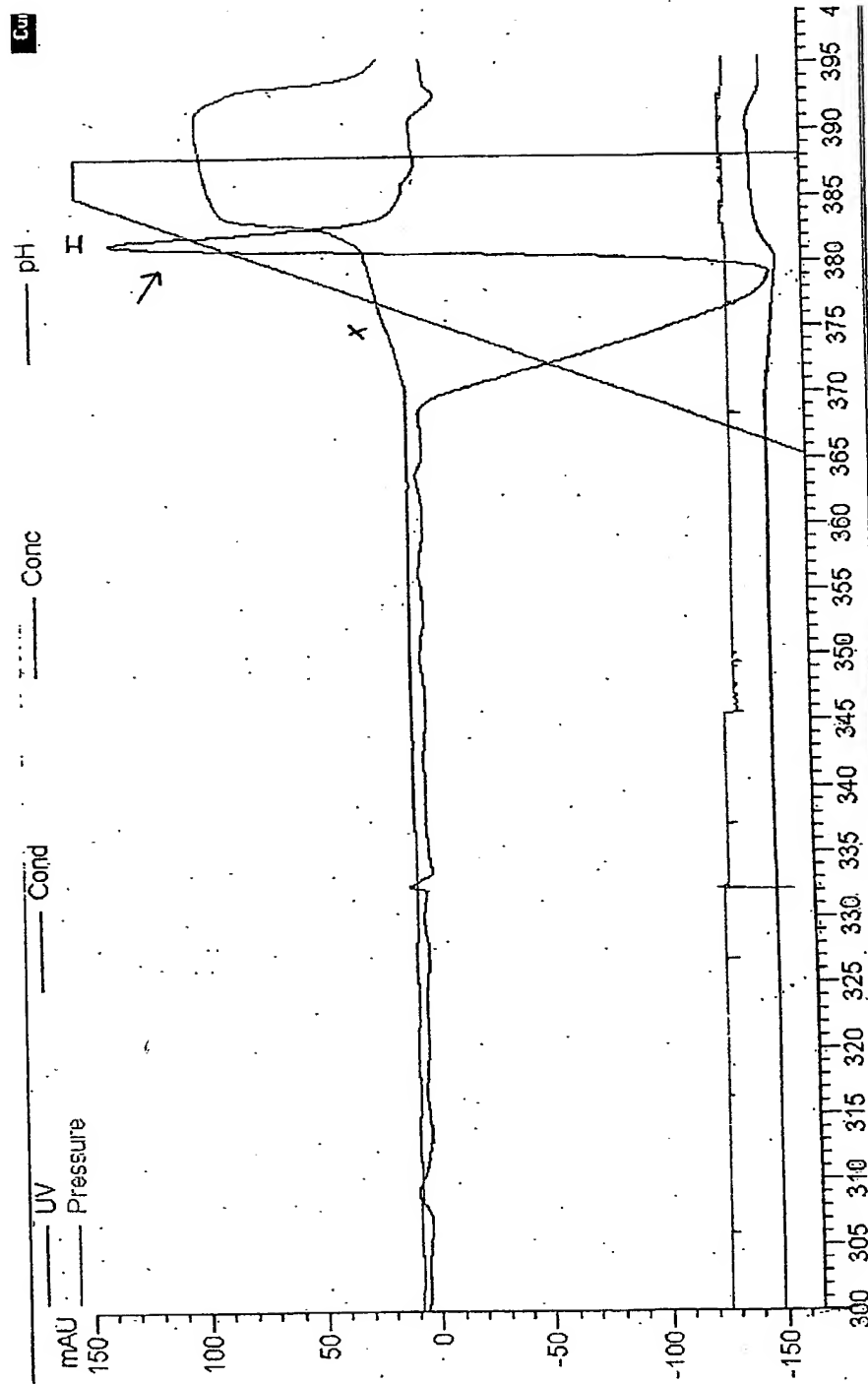


FIG. 3

FIG. 4

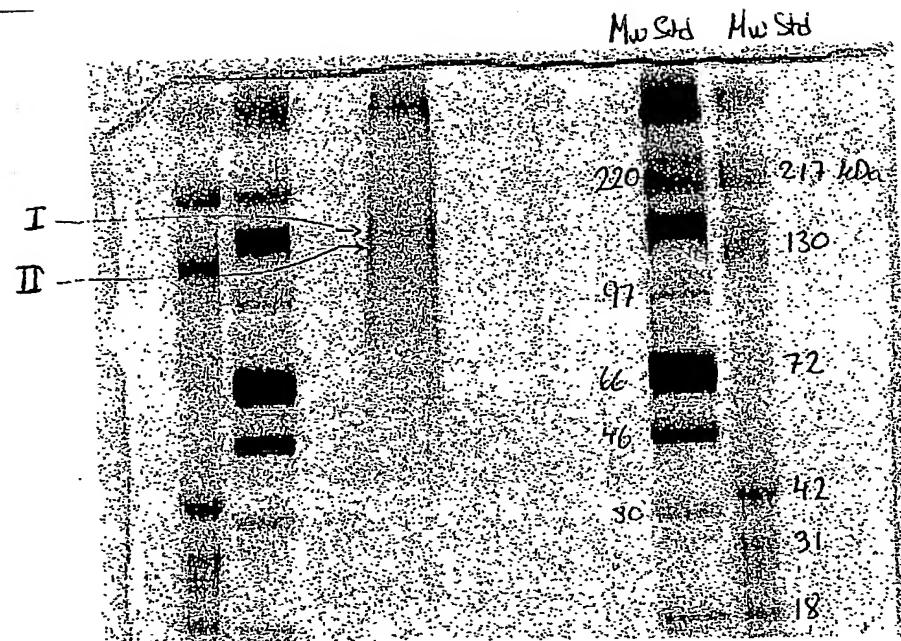


FIG. 5A

TA6-Human integrin ALPHA-6A

MAAAGQLCLLYLSAGLLSRLGAAFNLDTREDNVIRKYGDPGSLFGFSLAMHWQLQP
EDKRLLLVGAPRGEALPLQRANRTGGLYSCDITARGPCTRIEFDNDADPTSESKEDQ
WMGVTVQSQGPGGKVVTCAHRYEKRQHVNTKQESRDIFGRCYVLSQNLRIEDDMD
GGDWSFCDGRLRGHEKFGSCQOQGVAAATFTKDFHYIVFGAPGTYNWKGIVRVEQKN
NTFFDMNIFEDGPYEVGGETEHDESLVPVPANSYLGFSLD SGKGIVSKDEITFVSGAPR
ANHSGAVVLLKRDMKSAHLLPEHIFDGEGLASSFGYDVA VVDLNKDGWODIVIGAP
QYFDRDGEVGGAVYVYMNOQGRWNNVKPIRLNGTKDSMFGIAVK NIGDINODGY P
DIAVGAPYDDLGKVFIYHGSANGINTKPTQVLKGISPYFGYSIAGNMDLDRNSYPDV
AVGSLSDSVTIFRSRPVINIQKTITVTPNRIDLRQKTACGAPSGICLQVKSCFEYTANPA
GYNPSISIVGTLEAEKERRKSGLSSRVQFRNQGSEP KYTQELTLKRQKQKVCMEETL
WLQDNIRDKL RPIPITASVEIQEPSSRRRVNSLPEVLPILNSDEPKTAHIDVHFLKEGCG
DDNVCNSNLKLEYKFCTREGNQDKFSYLP IQKGVPPELV LKDQKDIALEITVTNPSNP
RNPTKDGDDAHEAKLIATFPDTLTYSAYRELRAFPEKQLSCVANQNGSQADCELGNP
FKRNSNVTFYLV LSTTEVTFDTPDLINLKLETTSNQDN LAPITAKAKVVIELLLSVSG
VAKPSQVYFGGTVVGEQAMKSEDEVGSLIEYEFRVINLGKPLTNLGTATLNIQWPKEI
SNGKWLLYL VKVESKGLEKVTCEPOKEINSLNLTESHNSRKKREITEKQIDDN RKFSL
FAERKYOTLNCSVNVNCVNIRCPLRGLDSKASLILRSRLWNSTFLEEYSKLN YLDILM
RAFIDVTAAAENIRLPNAGTQVRVTVPFSKTVAQYSGVPWWIILVAILAGILMLALLV
FILWKCGFFKR NKKDHYDATYHKA EIHAQPSDKERLTSDA

FIG. 5B

INTEGRIN BETA-4 (PRECURSOR)

MAGPRPSPWARLLLAALISVSLSGTLANRCKKAPVKSCTECVRVKDKCAYCTDEMF
RDRRCNTQAELLAAGCQRESIVMESSFQITEETQIDTTLRSSQMSPQGLRVRLRPG
ERHFELEVFEPLSPVDLYILMDFSNSMSDDLNLKMGQNLARVLSQLTSDYTIGFG
KFVDKVSVPOTDMRPEKLKEPWPNSDPFSEKENVISLTEDVDEFNKLQGERISGNLD
APEGGFDAILQTAVCTRDIGWRPDSTHLLVFSTESAFHYEADGANVLAGIMSRNDER
CHLDTTGTYTQYRTQDYPSVPTLVRLAKHNIPIFAVTNYSYSYIEKLHTYFPVSSLG
VLQEDSSNIVELLEAFNRIRSNLDIRALDSPRGLRTEVTSKMFQKTRTGSFHRRGEV
GIYQVQLRALEHVDGTHVCOLPEDOKGNIHLKPSFSDGLKMDAGIICDVCTCELOKE
VRSARCSFNGDFVCGQCVCSEGWSGQTCNCSTGSLSDIQCLREGEDKPCSGRGECQ
CGHCVCYGEGRYEGQFCEYDNFQCPRTSGFLCNDRGRCSMGQCVCEPGWTGPSCDC
PLSNATCIDSNGGICNGRGHCECGRCHCHQQSLYTDITICEINYSAIHPGLCEDLRSCVQ
CQAWGTGEKKGRTCEEKNFKVKMVDELKRAEEVVVRCSFRDEDDDDCTYSYTMEDG
GAPGPNSTVLVHKKKDCPPGSFWWLPLLLLLLPLALLLLLCWKYCACCKACLALL
PCCNRGHMVGFKEDHYMLRENLMASDHLDTPMLRSGNLKGRDVVRWKVTNNMQR
PGFATHAASINPTELVPYGLSLRLARLCTENLLKPDTRCAQLRQEVEENLNEVYRQI
SGVHKLQQTFRQPNAGKKQDHTIVDTVLMAPRSAKPALLKLTEKQVEQRAFDL
KVAPGYTTLTADODARGMVEFQEGVELVDVRVPLFIRPEDDDEKQLLVEAIDVPAG
TATLGRRLVNITIIKEQARDVVSFEQPEFSVSRGDQVARIPVIRRVLDGGKSQVSYRTQ
DGTAQGNRDYIPVEGELLFQPGEA WKELQVKLELOEVDSSLRGRQVRRFHVQLSNP
KFGAHLGQPHSTTHIRDPDELDRSFTSQMLSSQPPPHGDLGAPQNPNAKAAGSRKIH
NWLPPSGKPMGYRVKYWIQGDSESEAHLLDSKVPSVELTNLYPYCDYEMKVCAYG
AQEGEPYSSLVSCRTHQEVPSPEGRLAFNVVSSTVTQLSWAEPATNGEITAYEVCY
GLVNDDNRPIGPMKKVLVDNPKNRMILLIENLRESQPYRYTVKARNAGWGPEREAI
NLATQPKRPMSPPIPDPIVDAQSGEDYDSFLMYSDDVLRSPSGSQRPVSDDTGCGW
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SATPGPPGEHLVNGRMDFAFPGSTNSLHRMTTTSAAAYGTHLSPHVPHRVLSTSSTLT
RDYNSLTRSEHSHSTTLPRDYSTLTSVSSHGLPPIWEHGRSRLPLSWALGSRRAQMK
GFPPSRGPRDSIILAGRPAAPSWGPD SRLTAGVPDTPTRLVFSALGPTSLRVSWQEPRC
ERPLQGY SVEYQLLNGGELHRLNPNPAQTSVVVEDLLPNHSYVFRVRAQSQEGWGR
EREGVITIESQVHPQSPLCPLPGSAFTLSTPSAPGPLVFTALSPDSLQLSWERPRRPN
IVGYLVTCEMAQGGGPATAFRVDGDSPE SRLTVPGLSENVYPYKFKVQARTTEGFGPE
REGIITIESQDGGPFPQLGSRAGLFQHPLQSEYSSITTTHTSATEPFLVDGP TLGAQHLE
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FIG. 6

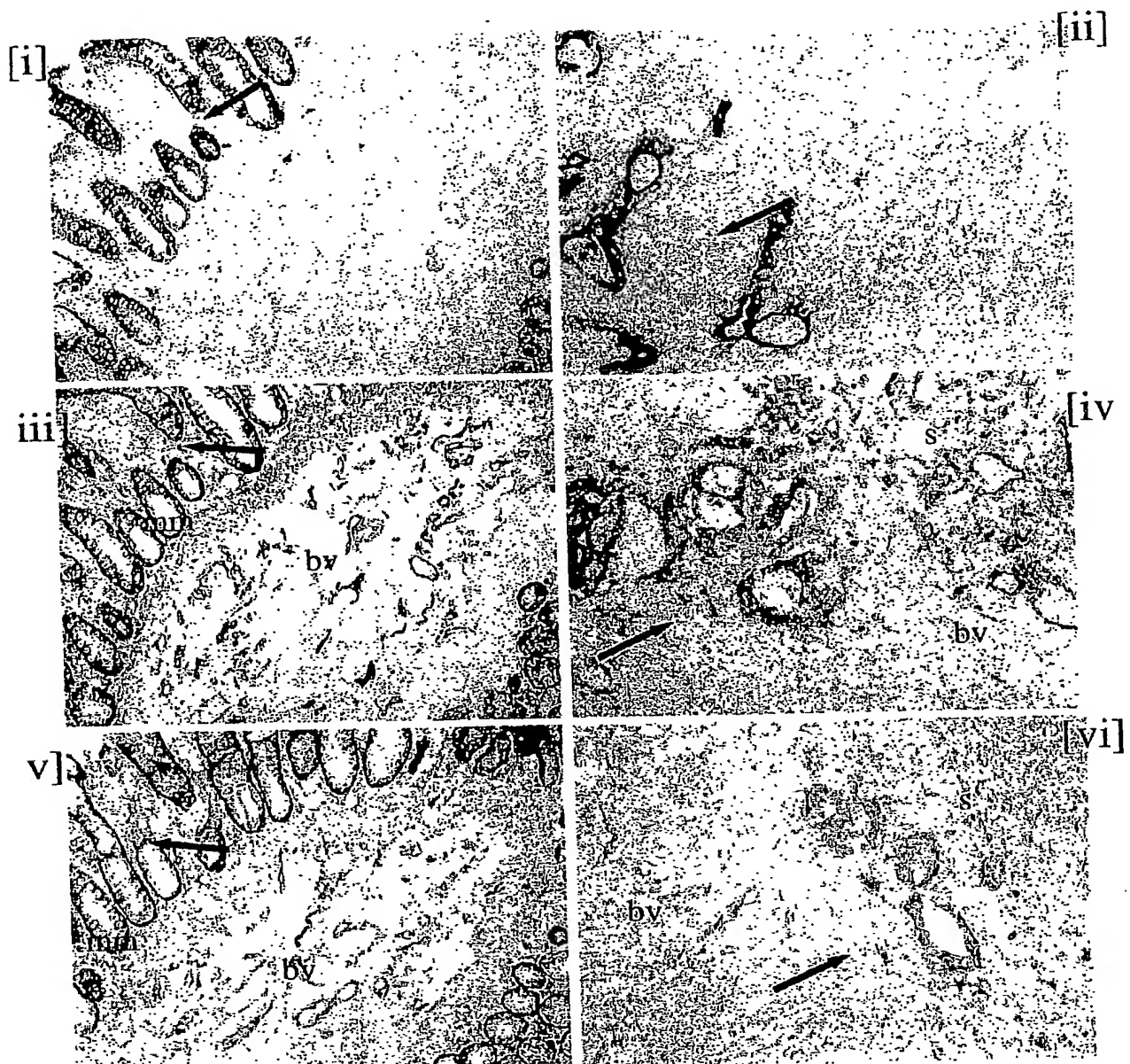


FIG. 7A

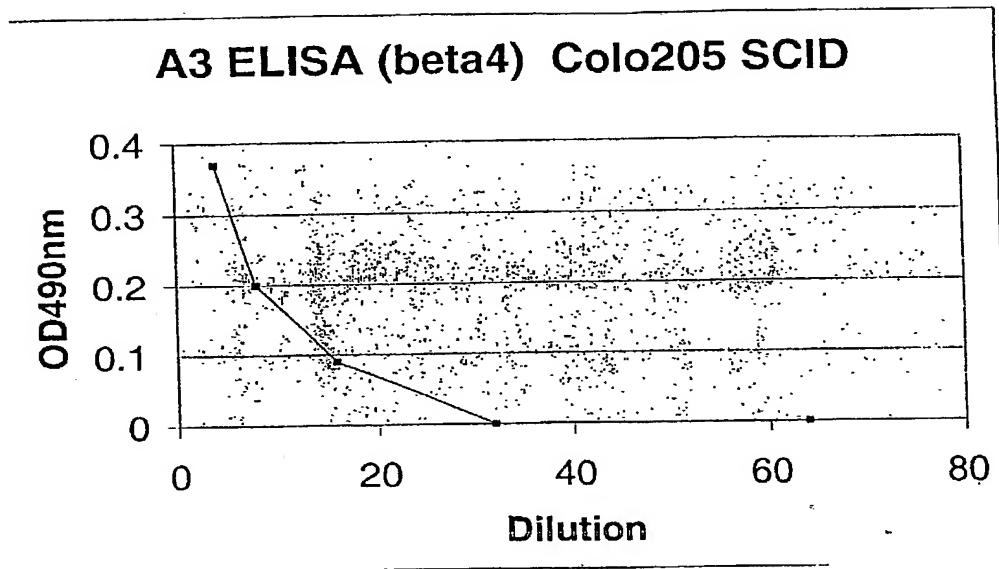


FIG. 7B

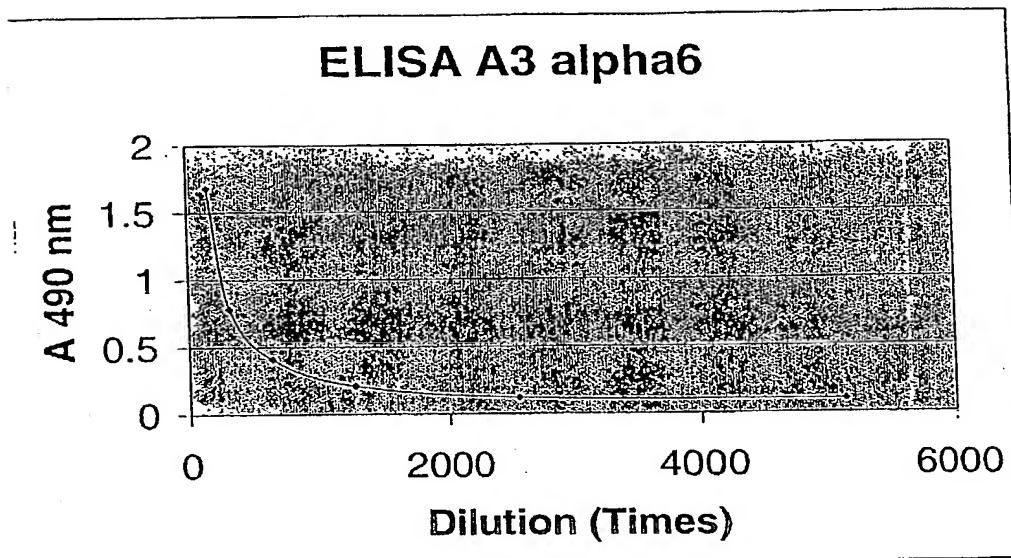
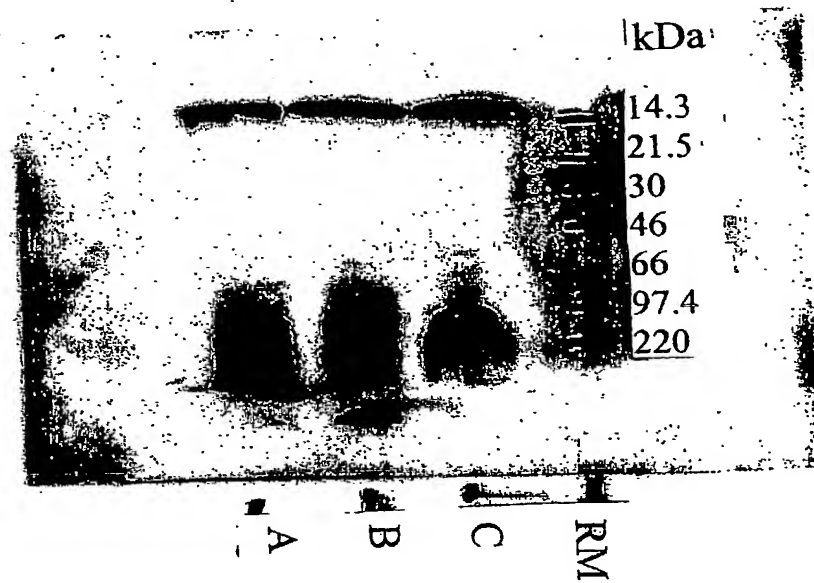


FIG. 8A
(i)

scFvA3FabSEAm9 (4 µg/ml)

αSEA-HRP (1/2000)



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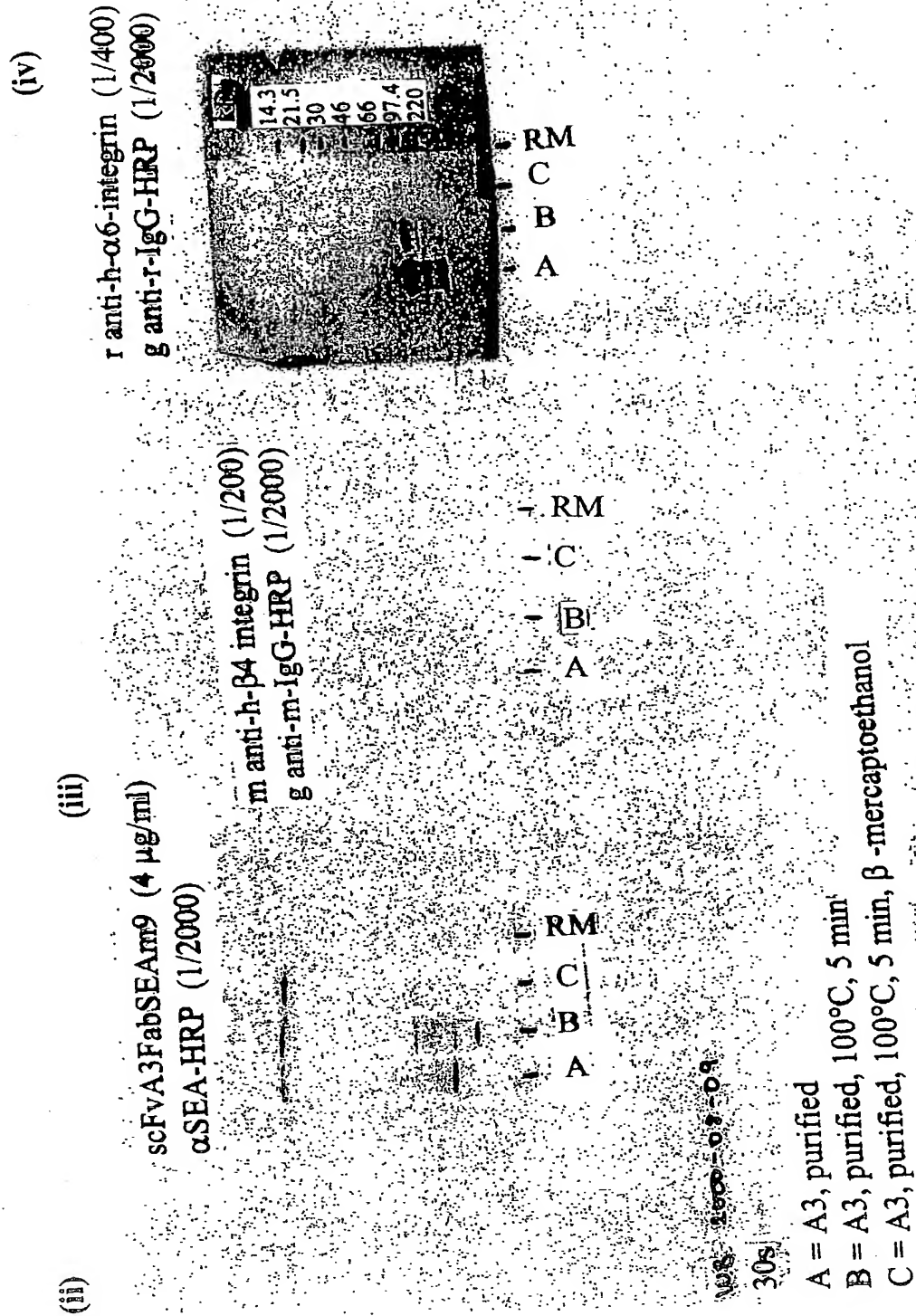


FIG. 8B

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and International (PCT) Applications)Attorney's Docket
No. 003300-920

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (IF ONLY ONE NAME IS LISTED BELOW) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (IF PLURAL NAMES ARE LISTED BELOW) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

NOVEL COMPOUNDS

The specification of which (check only one item below):

- ☐ is attached hereto.
- ☐ was filed as United States Patent Application Number _____
on _____
and was amended on _____ (if applicable).
- ☒ was filed as International (PCT) Application Number SE00/02082
on 26 October 2000
and was amended on _____ (if applicable).

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE U.S. PATENT AND TRADEMARK OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than six months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code, §§ 119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any International (PCT) Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International (PCT) Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Sweden	9903895-2	28 October 1999	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
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			<input type="checkbox"/> Yes <input type="checkbox"/> No
			<input type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

(APPLICATION NUMBER)

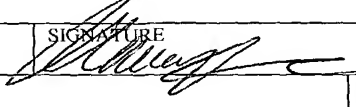
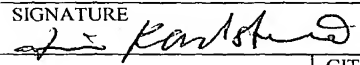
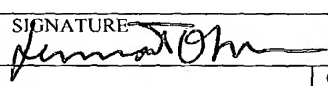
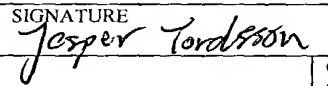
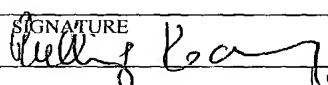
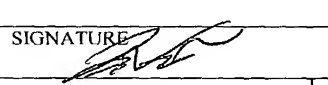
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(APPLICATION NUMBER)

(FILING DATE)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D)
(Includes Reference to Provisional and International (PCT) Applications)

Attorney's Docket
No. 003300-920

1-00	FULL NAME OF SOLE OR FIRST INVENTOR <u>Thomas BRODIN</u>	SIGNATURE 	DATE 13 February 2002
	RESIDENCE (CITY & STATE/COUNTRY) <u>RÅÅ, Sweden</u> SEX	CITIZENSHIP Swedish	
	POST OFFICE ADDRESS (HOME ADDRESS) <u>Lidängsgatan 10, 252 71 RÅÅ, SWEDEN</u>		
1-00	FULL NAME OF SECOND JOINT INVENTOR, IF ANY <u>Pia J. KARLSTRÖM</u>	SIGNATURE 	DATE 11 February 2002
	RESIDENCE (CITY & STATE/COUNTRY) <u>Lund, Sweden</u> SEX	CITIZENSHIP Swedish	
	POST OFFICE ADDRESS (HOME ADDRESS) <u>Fjellievägen 10A, 227 36 LUND, SWEDEN</u>		
3-00	FULL NAME OF THIRD JOINT INVENTOR, IF ANY <u>Lennart G. OHLSSON</u>	SIGNATURE 	DATE 12 February 2002
	RESIDENCE (CITY & STATE/COUNTRY) <u>Lund, Sweden</u> SEX	CITIZENSHIP Swedish	
	POST OFFICE ADDRESS (HOME ADDRESS) <u>Rudeboksvägen 898, 226 55 LUND, SWEDEN</u>		
4-00	FULL NAME OF FOURTH JOINT INVENTOR, IF ANY <u>Jesper M. TORDSSON</u>	SIGNATURE 	DATE 12 February 2002
	RESIDENCE (CITY & STATE/COUNTRY) <u>Lund, Sweden</u> SEX	CITIZENSHIP Swedish	
	POST OFFICE ADDRESS (HOME ADDRESS) <u>Iliongränden K120, 224 74 LUND, SWEDEN</u>		
5-00	FULL NAME OF FIFTH JOINT INVENTOR, IF ANY <u>Philip P. KEARNEY</u>	SIGNATURE 	DATE 12 February 2002
	RESIDENCE (CITY & STATE/COUNTRY) <u>Lund, Sweden</u> SEX	CITIZENSHIP Australian	
	POST OFFICE ADDRESS (HOME ADDRESS) <u>Kulgränden 15C, 226 49 LUND, SWEDEN</u>		
6-00	FULL NAME OF SIXTH JOINT INVENTOR, IF ANY <u>Bo H. K. Nilson</u>	SIGNATURE 	DATE 11 February 2002
	RESIDENCE (CITY & STATE/COUNTRY) <u>Lund, Sweden</u> SEX	CITIZENSHIP Swedish	
	POST OFFICE ADDRESS (HOME ADDRESS) <u>Sölvegatan 11, 223 62 LUND, SWEDEN</u>		
	FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
	RESIDENCE (CITY & STATE/COUNTRY)	CITIZENSHIP	
	POST OFFICE ADDRESS (HOME ADDRESS)		
	FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
	RESIDENCE (CITY & STATE/COUNTRY)	CITIZENSHIP	
	POST OFFICE ADDRESS (HOME ADDRESS)		
	FULL NAME OF NINTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
	RESIDENCE (CITY & STATE/COUNTRY)	CITIZENSHIP	
	POST OFFICE ADDRESS (HOME ADDRESS)		
	FULL NAME OF TENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
	RESIDENCE (CITY & STATE/COUNTRY)	CITIZENSHIP	
	POST OFFICE ADDRESS (HOME ADDRESS)		

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PCT/SE00/02082
JC13 Rec'd PCT/PTO 20 MAR 2002

SEQUENCE LISTING

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<151> 1999-10-28

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Gly Asn Asn Tyr Arg Pro Ser Gly Ile Pro Gly Arg Phe Ser Gly Ser	
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Trp Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Ala Ala Gln Val Glu	
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Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Trp Asp Ser Ser Gly Thr His	
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ccg gta ttc ggc gga ggg acc cgg gtg acc gtc cta ggt caa gcc aac	336
Pro Val Phe Gly Gly Gly Thr Arg Val Thr Val Leu Gly Gln Ala Asn	
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1008639 .032002

#6

Patent
Attorney's Docket No. 003300-920

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)	
)	
Thomas BRODIN <i>et al.</i>)	Group Art Unit: Not yet assigned
)	
Serial No.: 10/088,639)	Examiner: Not yet assigned
)	
Filed: March 20, 2002)	Confirmation No.: 7152
)	
For: NOVEL COMPOUNDS)	ATTENTION: BOX SEQUENCE

DECLARATION PURSUANT TO
37 C.F.R. §§ 1.821-1.825

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Deborah H. Yellin, declare as follows:

1. That the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same in compliance with § 1.821(f).
2. That the submission, filed in accordance with 37 C.F.R. § 1.821(g)[or (h)], herein does not include new matter [or go beyond the disclosure in the international application].
3. That the substitute copy of the computer readable form, submitted in accordance with 37 C.F.R. § 1.825(d), is identical to that originally filed.

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PCT/SE00/02082

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 35 40 45

Gly Asn Asn Tyr Arg Pro Ser Gly Ile Pro Gly Arg Phe Ser Gly Ser
 50 55 60

Trp Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Ala Ala Gln Val Glu
 65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Trp Asp Ser Ser Gly Thr His
 85 90 95

Pro Val Phe Gly Gly Gly Thr Arg Val Thr Val Leu Gly Gln Ala Asn
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Gly Glu Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
 115 120 125

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser
 130 135 140

Leu Arg Leu Ser Cys Val Ala Ser Gly Ser Ile Phe Ser Ser Ser Val
 145 150 155 160

Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
 165 170 175

Val Ile Ser Glu Asn Gly Arg Thr Ile Asn Tyr Ala Asp Ser Val Lys
 180 185 190

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Phe Leu
 195 200 205

Gln Met Asn Ser Leu Thr Gly Glu Asp Thr Ala Val Tyr Tyr Cys Ser
 210 215 220

Arg Glu Gly Gly Pro Gly Thr Thr Ser Asn Arg Leu Asp Ala Trp Gly
 225 230 235 240

Pro Gly Val Leu Val Thr Val Ser Ser
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Val Ile Arg Lys Tyr Gly Asp Pro Gly Ser Leu Phe Gly Phe Ser Leu
 35 40 45

Ala Met His Trp Gln Leu Gln Pro Glu Asp Lys Arg Leu Leu Leu Val
 50 55 60

Gly Ala Pro Arg Gly Glu Ala Leu Pro Leu Gln Arg Ala Asn Arg Thr
 65 70 75 80

Gly Gly Leu Tyr Ser Cys Asp Ile Thr Ala Arg Gly Pro Cys Thr Arg
 85 90 95

Ile Glu Phe Asp Asn Asp Ala Asp Pro Thr Ser Glu Ser Lys Glu Asp
 100 105 110

Gln Trp Met Gly Val Thr Val Gln Ser Gln Gly Pro Gly Gly Lys Val
 115 120 125

5

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Ile Asn Leu Lys Leu Glu Thr Thr Ser Asn Gln Asp Asn Leu Ala Pro
 770 775 780
 Ile Thr Ala Lys Ala Lys Val Val Ile Glu Leu Leu Leu Ser Val Ser
 785 790 795 800
 Gly Val Ala Lys Pro Ser Gln Val Tyr Phe Gly Gly Thr Val Val Gly
 805 810 815
 Glu Gln Ala Met Lys Ser Glu Asp Glu Val Gly Ser Leu Ile Glu Tyr
 820 825 830
 Glu Phe Arg Val Ile Asn Leu Gly Lys Pro Leu Thr Asn Leu Gly Thr
 835 840 845
 Ala Thr Leu Asn Ile Gln Trp Pro Lys Glu Ile Ser Asn Gly Lys Trp
 850 855 860
 Leu Leu Tyr Leu Val Lys Val Glu Ser Lys Gly Leu Glu Lys Val Thr
 865 870 875 880
 Cys Glu Pro Gln Lys Glu Ile Asn Ser Leu Asn Leu Thr Glu Ser His
 885 890 895
 Asn Ser Arg Lys Lys Arg Glu Ile Thr Glu Lys Gln Ile Asp Asp Asn
 900 905 910
 Arg Lys Phe Ser Leu Phe Ala Glu Arg Lys Tyr Gln Thr Leu Asn Cys
 915 920 925
 Ser Val Asn Val Asn Cys Val Asn Ile Arg Cys Pro Leu Arg Gly Leu
 930 935 940
 Asp Ser Lys Ala Ser Leu Ile Leu Arg Ser Arg Leu Trp Asn Ser Thr
 945 950 955 960
 Phe Leu Glu Glu Tyr Ser Lys Leu Asn Tyr Leu Asp Ile Leu Met Arg
 965 970 975
 Ala Phe Ile Asp Val Thr Ala Ala Ala Glu Asn Ile Arg Leu Pro Asn
 980 985 990
 Ala Gly Thr Gln Val Arg Val Thr Val Phe Pro Ser Lys Thr Val Ala
 995 1000 1005
 Gln Tyr Ser Gly Val Pro Trp Trp Ile Ile Leu Val Ala Ile Leu Ala
 1010 1015 1020
 Gly Ile Leu Met Leu Ala Leu Leu Val Phe Ile Leu Trp Lys Cys Gly
 1025 1030 1035 1040
 Phe Phe Lys Arg Asn Lys Lys Asp His Tyr Asp Ala Thr Tyr His Lys
 1045 1050 1055
 Ala Glu Ile His Ala Gln Pro Ser Asp Lys Glu Arg Leu Thr Ser Asp
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<210> 4

<211> 1875

<212> PRT

<213> Human

<223> Integrin beta-4 (precursor)

<400> 4

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Ala Pro Val Lys Ser Cys Thr Glu Cys Val Arg Val Asp Lys Asp Cys
      35              40              45

Ala Tyr Cys Thr Asp Glu Met Phe Arg Asp Arg Arg Cys Asn Thr Gln
      50              55              60

Ala Glu Leu Leu Ala Ala Gly Cys Gln Arg Glu Ser Ile Val Val Met
      65              70              75              80

Glu Ser Ser Phe Gln Ile Thr Glu Glu Thr Gln Ile Asp Thr Thr Leu
      85              90              95

Arg Arg Ser Gln Met Ser Pro Gln Gly Leu Arg Val Arg Leu Arg Pro
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Gly Glu Glu Arg His Phe Glu Leu Glu Val Phe Glu Pro Leu Glu Ser
      115             120             125

Pro Val Asp Leu Tyr Ile Leu Met Asp Phe Ser Asn Ser Met Ser Asp
      130             135             140

Asp Leu Asp Asn Leu Lys Lys Met Gly Gln Asn Leu Ala Arg Val Leu
      145             150             155             160

Ser Gln Leu Thr Ser Asp Tyr Thr Ile Gly Phe Gly Lys Phe Val Asp
      165             170             175

Lys Val Ser Val Pro Gln Thr Asp Met Arg Pro Glu Lys Leu Lys Glu
      180             185             190

Pro Trp Pro Asn Ser Asp Pro Pro Phe Ser Phe Lys Asn Val Ile Ser
      195             200             205

Leu Thr Glu Asp Val Asp Glu Phe Arg Asn Lys Leu Gln Gly Glu Arg
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Ile Ser Gly Asn Leu Asp Ala Pro Glu Gly Gly Phe Asp Ala Ile Leu
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Gln Thr Ala Val Cys Thr Arg Asp Ile Gly Trp Arg Pro Asp Ser Thr
      245             250             255

His Leu Leu Val Phe Ser Thr Glu Ser Ala Phe His Tyr Glu Ala Asp
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Gly Ala Asn Val Leu Ala Gly Ile Met Ser Arg Asn Asp Glu Arg Cys
      275             280             285

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His Leu Asp Thr Thr Gly Thr Tyr Thr Gln Tyr Arg Thr Gln Asp Tyr
 290 295 300

Pro Ser Val Pro Thr Leu Val Arg Leu Leu Ala Lys His Asn Ile Ile
 305 310 315 320

Pro Ile Phe Ala Val Thr Asn Tyr Ser Tyr Ser Tyr Tyr Glu Lys Leu
 325 330 335

His Thr Tyr Phe Pro Val Ser Ser Leu Gly Val Leu Gln Glu Asp Ser
 340 345 350

Ser Asn Ile Val Glu Leu Leu Glu Glu Ala Phe Asn Arg Ile Arg Ser
 355 360 365

Asn Leu Asp Ile Arg Ala Leu Asp Ser Pro Arg Gly Leu Arg Thr Glu
 370 375 380

Val Thr Ser Lys Met Phe Gln Lys Thr Arg Thr Gly Ser Phe His Ile
 385 390 395 400

Arg Arg Gly Glu Val Gly Ile Tyr Gln Val Gln Leu Arg Ala Leu Glu
 405 410 415

His Val Asp Gly Thr His Val Cys Gln Leu Pro Glu Asp Gln Lys Gly
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Asn Ile His Leu Lys Pro Ser Phe Ser Asp Gly Leu Lys Met Asp Ala
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Gly Ile Ile Cys Asp Val Cys Thr Cys Glu Leu Gln Lys Glu Val Arg
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Ser Ala Arg Cys Ser Phe Asn Gly Asp Phe Val Cys Gly Gln Cys Val
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Cys Ser Glu Gly Trp Ser Gly Gln Thr Cys Asn Cys Ser Thr Gly Ser
 485 490 495

Leu Ser Asp Ile Gln Pro Cys Leu Arg Glu Gly Glu Asp Lys Pro Cys
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Ser Gly Arg Gly Glu Cys Gln Cys Gly His Cys Val Cys Tyr Gly Glu
 515 520 525

Gly Arg Tyr Glu Gly Gln Phe Cys Glu Tyr Asp Asn Phe Gln Cys Pro
 530 535 540

Arg Thr Ser Gly Phe Leu Cys Asn Asp Arg Gly Arg Cys Ser Met Gly
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Gln Cys Val Cys Glu Pro Gly Trp Thr Gly Pro Ser Cys Asp Cys Pro
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Leu Ser Asn Ala Thr Cys Ile Asp Ser Asn Gly Gly Ile Cys Asn Gly
 580 585 590

Arg Gly His Cys Glu Cys Gly Arg Cys His Cys His Gln Gln Ser Leu
 595 600 605

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Tyr Thr Asp Thr Ile Cys Glu Ile Asn Tyr Ser Ala Ile His Pro Gly
 610 615 620
 Leu Cys Glu Asp Leu Arg Ser Cys Val Gln Cys Gln Ala Trp Gly Thr
 625 630 635 640
 Gly Glu Lys Lys Gly Arg Thr Cys Glu Glu Cys Asn Phe Lys Val Lys
 645 650 655
 Met Val Asp Glu Leu Lys Arg Ala Glu Glu Val Val Val Arg Cys Ser
 660 665 670
 Phe Arg Asp Glu Asp Asp Asp Cys Thr Tyr Ser Tyr Thr Met Glu Gly
 675 680 685
 Asp Gly Ala Pro Gly Pro Asn Ser Thr Val Leu Val His Lys Lys Lys
 690 695 700
 Asp Cys Pro Pro Gly Ser Phe Trp Trp Leu Ile Pro Leu Leu Leu Leu
 705 710 715 720
 Leu Leu Pro Leu Leu Ala Leu Leu Leu Leu Leu Cys Trp Lys Tyr Cys
 725 730 735
 Ala Cys Cys Lys Ala Cys Leu Ala Leu Leu Pro Cys Cys Asn Arg Gly
 740 745 750
 His Met Val Gly Phe Lys Glu Asp His Tyr Met Leu Arg Glu Asn Leu
 755 760 765
 Met Ala Ser Asp His Leu Asp Thr Pro Met Leu Arg Ser Gly Asn Leu
 770 775 780
 Lys Gly Arg Asp Val Val Arg Trp Lys Val Thr Asn Asn Met Gln Arg
 785 790 795 800
 Pro Gly Phe Ala Thr His Ala Ala Ser Ile Asn Pro Thr Glu Leu Val
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 Pro Tyr Gly Leu Ser Leu Arg Leu Ala Arg Leu Cys Thr Glu Asn Leu
 820 825 830
 Leu Lys Pro Asp Thr Arg Glu Cys Ala Gln Leu Arg Gln Glu Val Glu
 835 840 845
 Glu Asn Leu Asn Glu Val Tyr Arg Gln Ile Ser Gly Val His Lys Leu
 850 855 860
 Gln Gln Thr Lys Phe Arg Gln Gln Pro Asn Ala Gly Lys Lys Gln Asp
 865 870 875 880
 His Thr Ile Val Asp Thr Val Leu Met Ala Pro Arg Ser Ala Lys Pro
 885 890 895
 Ala Leu Leu Lys Leu Thr Glu Lys Gln Val Glu Gln Arg Ala Phe His
 900 905 910
 Asp Leu Lys Val Ala Pro Gly Tyr Tyr Thr Leu Thr Ala Asp Gln Asp
 915 920 925

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Ala Arg Gly Met Val Glu Phe Gln Glu Gly Val Glu Leu Val Asp Val
930 935 940

Arg Val Pro Leu Phe Ile Arg Pro Glu Asp Asp Asp Glu Lys Gln Leu
945 950 955 960

Leu Val Glu Ala Ile Asp Val Pro Ala Gly Thr Ala Thr Leu Gly Arg
965 970 975

Arg Leu Val Asn Ile Thr Ile Ile Lys Glu Gln Ala Arg Asp Val Val
980 985 990

Ser Phe Glu Gln Pro Glu Phe Ser Val Ser Arg Gly Asp Gln Val Ala
995 1000 1005

Arg Ile Pro Val Ile Arg Arg Val Leu Asp Gly Gly Lys Ser Gln Val
1010 1015 1020

Ser Tyr Arg Thr Gln Asp Gly Thr Ala Gln Gly Asn Arg Asp Tyr Ile
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Pro Val Glu Gly Glu Leu Leu Phe Gln Pro Gly Glu Ala Trp Lys Glu
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Leu Gln Val Lys Leu Leu Glu Leu Gln Glu Val Asp Ser Leu Leu Arg
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Gly Arg Gln Val Arg Arg Phe His Val Gln Leu Ser Asn Pro Lys Phe
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Gly Ala His Leu Gly Gln Pro His Ser Thr Thr Ile Ile Ile Arg Asp
1090 1095 1100

Pro Asp Glu Leu Asp Arg Ser Phe Thr Ser Gln Met Leu Ser Ser Gln
1105 1110 1115 1120

Pro Pro Pro His Gly Asp Leu Gly Ala Pro Gln Asn Pro Asn Ala Lys
1125 1130 1135

Ala Ala Gly Ser Arg Lys Ile His Phe Asn Trp Leu Pro Pro Ser Gly
1140 1145 1150

Lys Pro Met Gly Tyr Arg Val Lys Tyr Trp Ile Gln Gly Asp Ser Glu
1155 1160 1165

Ser Glu Ala His Leu Leu Asp Ser Lys Val Pro Ser Val Glu Leu Thr
1170 1175 1180

Asn Leu Tyr Pro Tyr Cys Asp Tyr Glu Met Lys Val Cys Ala Tyr Gly
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Ala Gln Gly Glu Gly Pro Tyr Ser Ser Leu Val Ser Cys Arg Thr His
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Gln Glu Val Pro Ser Glu Pro Gly Arg Leu Ala Phe Asn Val Val Ser
1220 1225 1230

Ser Thr Val Thr Gln Leu Ser Trp Ala Glu Pro Ala Glu Thr Asn Gly
1235 1240 1245

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Glu Ile Thr Ala Tyr Glu Val Cys Tyr Gly Leu Val Asn Asp Asp Asn
 1250 1255 1260

Arg Pro Ile Gly Pro Met Lys Lys Val Leu Val Asp Asn Pro Lys Asn
 1265 1270 1275 1280

Arg Met Leu Leu Ile Glu Asn Leu Arg Glu Ser Gln Pro Tyr Arg Tyr
 1285 1290 1295

Thr Val Lys Ala Arg Asn Gly Ala Gly Trp Gly Pro Glu Arg Glu Ala
 1300 1305 1310

Ile Ile Asn Leu Ala Thr Gln Pro Lys Arg Pro Met Ser Ile Pro Ile
 1315 1320 1325

Ile Pro Asp Ile Pro Ile Val Asp Ala Gln Ser Gly Glu Asp Tyr Asp
 1330 1335 1340

Ser Phe Leu Met Tyr Ser Asp Asp Val Leu Arg Ser Pro Ser Gly Ser
 1345 1350 1355 1360

Gln Arg Pro Ser Val Ser Asp Asp Thr Gly Cys Gly Trp Lys Phe Glu
 1365 1370 1375

Pro Leu Leu Gly Glu Glu Leu Asp Leu Arg Arg Val Thr Trp Arg Leu
 1380 1385 1390

Pro Pro Glu Leu Ile Pro Arg Leu Ser Ala Ser Ser Gly Arg Ser Ser
 1395 1400 1405

Asp Ala Glu Ala Pro Thr Ala Pro Arg Thr Thr Ala Ala Arg Ala Gly
 1410 1415 1420

Arg Ala Ala Ala Val Pro Arg Ser Ala Thr Pro Gly Pro Pro Gly Glu
 1425 1430 1435 1440

His Leu Val Asn Gly Arg Met Asp Phe Ala Phe Pro Gly Ser Thr Asn
 1445 1450 1455

Ser Leu His Arg Met Thr Thr Thr Ser Ala Ala Ala Tyr Gly Thr His
 1460 1465 1470

Leu Ser Pro His Val Pro His Arg Val Leu Ser Thr Ser Ser Thr Leu
 1475 1480 1485

Thr Arg Asp Tyr Asn Ser Leu Thr Arg Ser Glu His Ser His Ser Thr
 1490 1495 1500

Thr Leu Pro Arg Asp Tyr Ser Thr Leu Thr Ser Val Ser Ser His Gly
 1505 1510 1515 1520

Leu Pro Pro Ile Trp Glu His Gly Arg Ser Arg Leu Pro Leu Ser Trp
 1525 1530 1535

Ala Leu Gly Ser Arg Ser Arg Ala Gln Met Lys Gly Phe Pro Pro Ser
 1540 1545 1550

Arg Gly Pro Arg Asp Ser Ile Ile Leu Ala Gly Arg Pro Ala Ala Pro
 1555 1560 1565

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Ser Trp Gly Pro Asp Ser Arg Leu Thr Ala Gly Val Pro Asp Thr Pro
 1570 1575 1580

Thr Arg Leu Val Phe Ser Ala Leu Gly Pro Thr Ser Leu Arg Val Ser
 1585 1590 1595 1600

Trp Gln Glu Pro Arg Cys Glu Arg Pro Leu Gln Gly Tyr Ser Val Glu
 1605 1610 1615

Tyr Gln Leu Leu Asn Gly Gly Glu Leu His Arg Leu Asn Ile Pro Asn
 1620 1625 1630

Pro Ala Gln Thr Ser Val Val Val Glu Asp Leu Leu Pro Asn His Ser
 1635 1640 1645

Tyr Val Phe Arg Val Arg Ala Gln Ser Gln Glu Gly Trp Gly Arg Glu
 1650 1655 1660

Arg Glu Gly Val Ile Thr Ile Glu Ser Gln Val His Pro Gln Ser Pro
 1665 1670 1675 1680

Leu Cys Pro Leu Pro Gly Ser Ala Phe Thr Leu Ser Thr Pro Ser Ala
 1685 1690 1695

Pro Gly Pro Leu Val Phe Thr Ala Leu Ser Pro Asp Ser Leu Gln Leu
 1700 1705 1710

Ser Trp Glu Arg Pro Arg Arg Pro Asn Gly Asp Ile Val Gly Tyr Leu
 1715 1720 1725

Val Thr Cys Glu Met Ala Gln Gly Gly Gly Pro Ala Thr Ala Phe Arg
 1730 1735 1740

Val Asp Gly Asp Ser Pro Glu Ser Arg Leu Thr Val Pro Gly Leu Ser
 1745 1750 1755 1760

Glu Asn Val Pro Tyr Lys Phe Lys Val Gln Ala Arg Thr Thr Glu Gly
 1765 1770 1775

Phe Gly Pro Glu Arg Glu Gly Ile Ile Thr Ile Glu Ser Gln Asp Gly
 1780 1785 1790

Gly Pro Phe Pro Gln Leu Gly Ser Arg Ala Gly Leu Phe Gln His Pro
 1795 1800 1805

Leu Gln Ser Glu Tyr Ser Ser Ile Thr Thr Thr His Thr Ser Ala Thr
 1810 1815 1820

Glu Pro Phe Leu Val Asp Gly Pro Thr Leu Gly Ala Gln His Leu Glu
 1825 1830 1835 1840

Ala Gly Gly Ser Leu Thr Arg His Val Thr Gln Glu Phe Val Ser Arg
 1845 1850 1855

Thr Leu Thr Thr Ser Gly Thr Leu Ser Thr His Met Asp Gln Gln Phe
 1860 1865 1870

Phe Gln Thr
 1875

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<211> 8
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<223> Amino acids 61-68 of SEQ ID NO: 3

<400> 5
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<210> 6
<211> 20
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Pro Cys Thr Arg
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<223> Amino acids 128-137 of SEQ ID NO: 3

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<210> 8
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<223> Amino acids 138-144 of SEQ ID NO: 3

<400> 8
Arg Gln His Val Asn Thr Lys
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<210> 9
<211> 9
<212> PRT
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<223> Amino acids 154-162 of SEQ ID NO: 3

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Cys Tyr Val Leu Ser Gln Asn Leu Arg
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<210> 10
<211> 14
<212> PRT
<213> Human

<223> Amino acids 185-198 of SEQ ID NO: 3

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<210> 11
<211> 16
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<223> Amino acids 199-214 of SEQ ID NO: 3

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<210> 12
<211> 11
<212> PRT
<213> Human

<223> Amino acids 272-282 of SEQ ID NO: 3

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<210> 13
<211> 11
<212> PRT
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<223> Amino acids 283-293 of SEQ ID NO: 3

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Ala Asn His Ser Gly Ala Val Val Leu Leu Lys
1 5 10

<210> 14
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<212> PRT
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<223> Amino acids 328-343 of SEQ ID NO: 3

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<210> 19
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 <213> Human

<223> Amino acids 445-463 of SEQ ID NO: 3

<400> 19
 Asn Ser Tyr Pro Asp Val Ala Val Gly Ser Leu Ser Asp Ser Val Thr
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Ile Phe Arg

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 <212> PRT
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<223> Amino acids 464-472 of SEQ ID NO: 3

<400> 20
 Ser Arg Pro Val Ile Asn Ile Gln Lys
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<210> 21
 <211> 18
 <212> PRT
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<223> Amino acids 577-594 of SEQ ID NO: 3

<400> 21
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Ser Arg

<210> 22
 <211> 17
 <212> PRT
 <213> Human

<223> Amino acids 597-613 of SEQ ID NO: 3

<400> 22
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Lys

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<223> Amino acids 968-976 of SEQ ID NO: 3

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<210> 32
 <211> 13
 <212> PRT
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<223> Amino acids 977-989 of SEQ ID NO: 3

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<210> 33
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<223> Amino acids 990-998 of SEQ ID NO: 3

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<210> 34
<211> 12
<212> PRT
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<210> 35
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<223> Amino acids 192-204 of SEQ ID NO: 4

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<210> 36
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<210> 37
<211> 12
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<223> Amino acids 301-312 of SEQ ID NO: 4

<400> 37
Thr Gln Asp Tyr Pro Ser Val Pro Thr Leu Val Arg
1 5 10

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PCT/SE00/02082

<210> 38
 <211> 12
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<223> Amino acids 402-413 of SEQ ID NO: 4

<400> 38
 Arg Gly Glu Val Gly Ile Tyr Gln Val Gln Leu Arg
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<210> 39
 <211> 18
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<223> Amino acids 414-431 of SEQ ID NO: 4

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Gln Lys

<210> 40
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<223> Amino acids 432-445 of SEQ ID NO: 4

<400> 40
 Gly Asn Ile His Leu Lys Pro Ser Phe Ser Asp Gly Leu Lys
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<210> 41
 <211> 16
 <212> PRT
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<223> Amino acids 446-461 of SEQ ID NO: 4

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<223> Amino acids 531-545 of SEQ ID NO: 4

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PCT/SE00/02082

<210> 43
<211> 16
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<223> Amino acids 631-646 of SEQ ID NO: 4

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PCT/SE00/02082

<210> 47
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<223> Amino acids 946-958 of SEQ ID NO: 4

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<210> 48
 <211> 14
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<223> Amino acids 990-1003 of SEQ ID NO: 4

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 Asp Val Val Ser Phe Glu Gln Pro Glu Phe Ser Val Ser Arg
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<210> 49
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<223> Amino acids 1061-1072 of SEQ ID NO: 4

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<210> 50
 <211> 19
 <212> PRT
 <213> Human

<223> Amino acids 1196-1214 of SEQ ID NO: 4

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Ser Cys Arg

<210> 51
 <211> 9
 <212> PRT
 <213> Human

<223> Amino acids 1273-1281 of SEQ ID NO: 4

<400> 51
 Val Leu Val Asp Asn Pro Lys Asn Arg
 1 5



003300-920.ST25

1003895.29 032002

#6

SEQUENCE LISTING

<110> Brodin, Thomas
Karlstrom, Pia J.
Ohlsson, Lennart G.
Tordsson, Jesper M.
Kearney, Philip P.
Nilson, Bo H.K.

<120> Novel Compounds

<130> 003300-920

<140> US 10/088,639

<141> 2002-03-20

<150> SE 9903895-2

<151> 1999-10-28

<160> 51

<170> PatentIn Ver. 2.1

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Thr	Val	Arg	Met	Thr	Cys	Gln	Gly	Asp	Ser	Leu	Lys	Thr	Tyr	Tyr	Ala	
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Ser	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Val	Pro	Val	Leu	Val	Ile	Tyr	
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Gly	Asn	Asn	Tyr	Arg	Pro	Ser	Gly	Ile	Pro	Gly	Arg	Phe	Ser	Gly	Ser	
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Trp	Ser	Gly	Asn	Thr	Ala	Ser	Leu	Thr	Ile	Thr	Ala	Ala	Gln	Val	Glu	
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Val Ile Arg Lys Tyr Gly Asp Pro Gly Ser Leu Phe Gly Phe Ser Leu
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Gly Ala Pro Arg Gly Glu Ala Leu Pro Leu Gln Arg Ala Asn Arg Thr
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Gly Gly Leu Tyr Ser Cys Asp Ile Thr Ala Arg Gly Pro Cys Thr Arg
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Ile Glu Phe Asp Asn Asp Ala Asp Pro Thr Ser Glu Ser Lys Glu Asp
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Gln Trp Met Gly Val Thr Val Gln Ser Gln Gly Pro Gly Gly Lys Val
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Gln Glu Ser Arg Asp Ile Phe Gly Arg Cys Tyr Val Leu Ser Gln Asn
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Leu Arg Ile Glu Asp Asp Met Asp Gly Gly Asp Trp Ser Phe Cys Asp
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Gly Arg Leu Arg Gly His Glu Lys Phe Gly Ser Cys Gln Gln Gly Val
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Ala Ala Thr Phe Thr Lys Asp Phe His Tyr Ile Val Phe Gly Ala Pro
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Gly Thr Tyr Asn Trp Lys Gly Ile Val Arg Val Glu Gln Lys Asn Asn
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Thr Phe Phe Asp Met Asn Ile Phe Glu Asp Gly Pro Tyr Glu Val Gly
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Gly Glu Thr Glu His Asp Glu Ser Leu Val Pro Val Pro Ala Asn Ser
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Tyr Leu Gly Phe Ser Leu Asp Ser Gly Lys Gly Ile Val Ser Lys Asp
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Glu Ile Thr Phe Val Ser Gly Ala Pro Arg Ala Asn His Ser Gly Ala
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Val Val Leu Leu Lys Arg Asp Met Lys Ser Ala His Leu Leu Pro Glu
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His Ile Phe Asp Gly Glu Gly Leu Ala Ser Ser Phe Gly Tyr Asp Val
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Ala Val Val Asp Leu Asn Lys Asp Gly Trp Gln Asp Ile Val Ile Gly
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Val Tyr Met Asn Gln Gln Gly Arg Trp Asn Asn Val Lys Pro Ile Arg
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Leu Asn Gly Thr Lys Asp Ser Met Phe Gly Ile Ala Val Lys Asn Ile
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Gly Asp Ile Asn Gln Asp Gly Tyr Pro Asp Ile Ala Val Gly Ala Pro
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Tyr Asp Asp Leu Gly Lys Val Phe Ile Tyr His Gly Ser Ala Asn Gly
405 410 415

Ile Asn Thr Lys Pro Thr Gln Val Leu Lys Gly Ile Ser Pro Tyr Phe
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Gly Tyr Ser Ile Ala Gly Asn Met Asp Leu Asp Arg Asn Ser Tyr Pro
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Asp Val Ala Val Gly Ser Leu Ser Asp Ser Val Thr Ile Phe Arg Ser
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Arg Pro Val Ile Asn Ile Gln Lys Thr Ile Thr Val Thr Pro Asn Arg
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Ile Asp Leu Arg Gln Lys Thr Ala Cys Gly Ala Pro Ser Gly Ile Cys
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Leu Gln Val Lys Ser Cys Phe Glu Tyr Thr Ala Asn Pro Ala Gly Tyr
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Glu Pro Lys Tyr Thr Gln Glu Leu Thr Leu Lys Arg Gln Lys Gln Lys
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Val Cys Met Glu Glu Thr Leu Trp Leu Gln Asp Asn Ile Arg Asp Lys
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Ser Asp Glu Pro Lys Thr Ala His Ile Asp Val His Phe Leu Lys Glu
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Lys Phe Cys Thr Arg Glu Gly Asn Gln Asp Lys Phe Ser Tyr Leu Pro
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Ile Gln Lys Gly Val Pro Glu Leu Val Leu Lys Asp Gln Lys Asp Ile
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Ala Leu Glu Ile Thr Val Thr Asn Ser Pro Ser Asn Pro Arg Asn Pro
675 680 685

Thr Lys Asp Gly Asp Asp Ala His Glu Ala Lys Leu Ile Ala Thr Phe
690 695 700

Pro Asp Thr Leu Thr Tyr Ser Ala Tyr Arg Glu Leu Arg Ala Phe Pro
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Glu Lys Gln Leu Ser Cys Val Ala Asn Gln Asn Gly Ser Gln Ala Asp
725 730 735

Cys Glu Leu Gly Asn Pro Phe Lys Arg Asn Ser Asn Val Thr Phe Tyr
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Leu Val Leu Ser Thr Thr Glu Val Thr Phe Asp Thr Pro Asp Leu Asp
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Ile Asn Leu Lys Leu Glu Thr Thr Ser Asn Gln Asp Asn Leu Ala Pro
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Ile Thr Ala Lys Ala Lys Val Val Ile Glu Leu Leu Leu Ser Val Ser
785 790 795 800

Gly Val Ala Lys Pro Ser Gln Val Tyr Phe Gly Gly Thr Val Val Gly
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Glu Gln Ala Met Lys Ser Glu Asp Glu Val Gly Ser Leu Ile Glu Tyr
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Glu Phe Arg Val Ile Asn Leu Gly Lys Pro Leu Thr Asn Leu Gly Thr
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Ala Thr Leu Asn Ile Gln Trp Pro Lys Glu Ile Ser Asn Gly Lys Trp
850 855 860

Leu Leu Tyr Leu Val Lys Val Glu Ser Lys Gly Leu Glu Lys Val Thr
865 870 875 880

Cys Glu Pro Gln Lys Glu Ile Asn Ser Leu Asn Leu Thr Glu Ser His
885 890 895

Asn Ser Arg Lys Lys Arg Glu Ile Thr Glu Lys Gln Ile Asp Asp Asn
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Arg Lys Phe Ser Leu Phe Ala Glu Arg Lys Tyr Gln Thr Leu Asn Cys
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 Gln Thr Ala Val Cys Thr Arg Asp Ile Gly Trp Arg Pro Asp Ser Thr
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 Pro Ser Val Pro Thr Leu Val Arg Leu Leu Ala Lys His Asn Ile Ile
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 Asn Ile His Leu Lys Pro Ser Phe Ser Asp Gly Leu Lys Met Asp Ala
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 Gly Ile Ile Cys Asp Val Cys Thr Cys Glu Leu Gln Lys Glu Val Arg
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 Cys Ser Glu Gly Trp Ser Gly Gln Thr Cys Asn Cys Ser Thr Gly Ser
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Leu Ser Asp Ile Gln Pro Cys Leu Arg Glu Gly Glu Asp Lys Pro Cys
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580 585 590

Arg Gly His Cys Glu Cys Gly Arg Cys His Cys His Gln Gln Ser Leu
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Tyr Thr Asp Thr Ile Cys Glu Ile Asn Tyr Ser Ala Ile His Pro Gly
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Leu Cys Glu Asp Leu Arg Ser Cys Val Gln Cys Gln Ala Trp Gly Thr
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Phe Arg Asp Glu Asp Asp Asp Cys Thr Tyr Ser Tyr Thr Met Glu Gly
675 680 685

Asp Gly Ala Pro Gly Pro Asn Ser Thr Val Leu Val His Lys Lys Lys
690 695 700

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Leu Leu Pro Leu Leu Ala Leu Leu Leu Leu Cys Trp Lys Tyr Cys
725 730 735

Ala Cys Cys Lys Ala Cys Leu Ala Leu Leu Pro Cys Cys Asn Arg Gly
740 745 750

His Met Val Gly Phe Lys Glu Asp His Tyr Met Leu Arg Glu Asn Leu
755 760 765

Met Ala Ser Asp His Leu Asp Thr Pro Met Leu Arg Ser Gly Asn Leu
770 775 780

Lys Gly Arg Asp Val Val Arg Trp Lys Val Thr Asn Asn Met Gln Arg
785 790 795 800

Pro Gly Phe Ala Thr His Ala Ala Ser Ile Asn Pro Thr Glu Leu Val
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Ala Ala Gly Ser Arg Lys Ile His Phe Asn Trp Leu Pro Pro Ser Gly
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 Lys Pro Met Gly Tyr Arg Val Lys Tyr Trp Ile Gln Gly Asp Ser Glu
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 Ser Glu Ala His Leu Leu Asp Ser Lys Val Pro Ser Val Glu Leu Thr
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 Asn Leu Tyr Pro Tyr Cys Asp Tyr Glu Met Lys Val Cys Ala Tyr Gly
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 Asp Ala Glu Ala Pro Thr Ala Pro Arg Thr Thr Ala Ala Arg Ala Gly
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Phe Gly Pro Glu Arg Glu Gly Ile Ile Thr Ile Glu Ser Gln Asp Gly
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 Gly Pro Phe Pro Gln Leu Gly Ser Arg Ala Gly Leu Phe Gln His Pro
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 Ala Gly Gly Ser Leu Thr Arg His Val Thr Gln Glu Phe Val Ser Arg
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 Thr Leu Thr Thr Ser Gly Thr Leu Ser Thr His Met Asp Gln Gln Phe
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Pro Cys Thr Arg
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SEQUENCE LISTING

<110> Brodin, Thomas
 Karlstrom, Pia J.
 Ohlsson, Lennart G.
 Tordsson, Jesper M.
 Kearney, Philip P.
 Nilson, Bo H.K.

<120> Novel Compounds

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<141> 2002-03-20

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 Ser Trp Tyr Gln Gln Lys Pro Gly Gln Val Pro Val Leu Val Ile Tyr
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 ggt aac aac tac cgg ccc tca ggg atc cca ggc cga ttc tct ggc tcc 192
 Gly Asn Asn Tyr Arg Pro Ser Gly Ile Pro Gly Arg Phe Ser Gly Ser
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 Trp Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Ala Ala Gln Val Glu
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Gly Glu Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Ser Glu
      115              120              125

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Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser
      130              135              140

ctg aga ctc tct tgt gta gcc tct ggg tcc atc ttc agt agc tct gtt 480
Leu Arg Leu Ser Cys Val Ala Ser Gly Ser Ile Phe Ser Ser Ser Val
      145              150              155

atg cac tgg gtc cgc cag gct cca gga aag ggt ctg gag tgg gtc tca 528
Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
      165              170              175

gtt att agt gaa aat ggg cgt acc att aac tac gca gac tct gtg aag 576
Val Ile Ser Glu Asn Gly Arg Thr Ile Asn Tyr Ala Asp Ser Val Lys
      180              185              190

ggc cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg ttt ctg 624
Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Phe Leu
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cag atg aac agc ctg aca ggc gag gac acg gcc gtc tat tac tgt agt 672
Gln Met Asn Ser Leu Thr Gly Glu Asp Thr Ala Val Tyr Tyr Cys Ser
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Arg Glu Gly Gly Pro Gly Thr Thr Ser Asn Arg Leu Asp Ala Trp Gly
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Ser Trp Tyr Gln Gln Lys Pro Gly Gln Val Pro Val Leu Val Ile Tyr
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Trp Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Ala Ala Gln Val Glu
65 70 75 80

Asp Glu Ala Asp Tyr Cys Asn Ser Trp Asp Ser Ser Gly Thr His
85 90 95

Pro Val Phe Gly Gly Gly Thr Arg Val Thr Val Leu Gly Gln Ala Asn
100 105 110

Gly Glu Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Glu
115 120 125

Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser
130 135 140

Leu Arg Leu Ser Cys Val Ala Ser Gly Ser Ile Phe Ser Ser Ser Val
145 150 155 160

Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser
165 170 175

Val Ile Ser Glu Asn Gly Arg Thr Ile Asn Tyr Ala Asp Ser Val Lys
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Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Phe Leu
195 200 205

Gln Met Asn Ser Leu Thr Gly Glu Asp Thr Ala Val Tyr Tyr Cys Ser
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 Gly Gly Leu Tyr Ser Cys Asp Ile Thr Ala Arg Gly Pro Cys Thr Arg
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 Ile Glu Phe Asp Asn Asp Ala Asp Pro Thr Ser Glu Ser Lys Glu Asp
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 260 265 270
 Glu Ile Thr Phe Val Ser Gly Ala Pro Arg Ala Asn His Ser Gly Ala
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 Val Val Leu Leu Lys Arg Asp Met Lys Ser Ala His Leu Leu Pro Glu
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 His Ile Phe Asp Gly Glu Gly Leu Ala Ser Ser Phe Gly Tyr Asp Val
 305 310 315 320
 Ala Val Val Asp Leu Asn Lys Asp Gly Trp Gln Asp Ile Val Ile Gly
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 Ala Pro Gln Tyr Phe Asp Arg Asp Gly Glu Val Gly Gly Ala Val Tyr
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 Val Tyr Met Asn Gln Gln Gly Arg Trp Asn Asn Val Lys Pro Ile Arg
 355 360 365

Leu Lys Pro Asp Thr Arg Glu Cys Ala Gln Leu Arg Gln Glu Val Glu
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 Ser Phe Glu Gln Pro Glu Phe Ser Val Ser Arg Gly Asp Gln Val Ala
 995 1000 1005
 Arg Ile Pro Val Ile Arg Arg Val Leu Asp Gly Gly Lys Ser Gln Val
 1010 1015 1020
 Ser Tyr Arg Thr Gln Asp Gly Thr Ala Gln Gly Asn Arg Asp Tyr Ile
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 Pro Val Glu Gly Glu Leu Leu Phe Gln Pro Gly Glu Ala Trp Lys Glu
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 Leu Gln Val Lys Leu Leu Glu Leu Gln Glu Val Asp Ser Leu Leu Arg
 1060 1065 1070
 Gly Arg Gln Val Arg Arg Phe His Val Gln Leu Ser Asn Pro Lys Phe
 1075 1080 1085
 Gly Ala His Leu Gly Gln Pro His Ser Thr Thr Ile Ile Ile Arg Asp
 1090 1095 1100
 Pro Asp Glu Leu Asp Arg Ser Phe Thr Ser Gln Met Leu Ser Ser Gln
 1105 1110 1115 1120
 Pro Pro Pro His Gly Asp Leu Gly Ala Pro Gln Asn Pro Asn Ala Lys
 1125 1130 1135
 Ala Ala Gly Ser Arg Lys Ile His Phe Asn Trp Leu Pro Pro Ser Gly
 1140 1145 1150

Lys Pro Met Gly Tyr Arg Val Lys Tyr Trp Ile Gln Gly Asp Ser Glu
1155 1160 1165

Ser Glu Ala His Leu Leu Asp Ser Lys Val Pro Ser Val Glu Leu Thr
1170 1175 1180

Asn Leu Tyr Pro Tyr Cys Asp Tyr Glu Met Lys Val Cys Ala Tyr Gly
1185 1190 1195 1200

Ala Gln Gly Glu Gly Pro Tyr Ser Ser Leu Val Ser Cys Arg Thr His
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Gln Glu Val Pro Ser Glu Pro Gly Arg Leu Ala Phe Asn Val Val Ser
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Ser Thr Val Thr Gln Leu Ser Trp Ala Glu Pro Ala Glu Thr Asn Gly
1235 1240 1245

Glu Ile Thr Ala Tyr Glu Val Cys Tyr Gly Leu Val Asn Asp Asp Asn
1250 1255 1260

Arg Pro Ile Gly Pro Met Lys Lys Val Leu Val Asp Asn Pro Lys Asn
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Arg Met Leu Leu Ile Glu Asn Leu Arg Glu Ser Gln Pro Tyr Arg Tyr
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Thr Val Lys Ala Arg Asn Gly Ala Gly Trp Gly Pro Glu Arg Glu Ala
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Ile Ile Asn Leu Ala Thr Gln Pro Lys Arg Pro Met Ser Ile Pro Ile
1315 1320 1325

Ile Pro Asp Ile Pro Ile Val Asp Ala Gln Ser Gly Glu Asp Tyr Asp
1330 1335 1340

Ser Phe Leu Met Tyr Ser Asp Asp Val Leu Arg Ser Pro Ser Gly Ser
1345 1350 1355 1360

Gln Arg Pro Ser Val Ser Asp Asp Thr Gly Cys Gly Trp Lys Phe Glu
1365 1370 1375

Pro Leu Leu Gly Glu Glu Leu Asp Leu Arg Arg Val Thr Trp Arg Leu
1380 1385 1390

Pro Pro Glu Leu Ile Pro Arg Leu Ser Ala Ser Ser Gly Arg Ser Ser
1395 1400 1405

Asp Ala Glu Ala Pro Thr Ala Pro Arg Thr Thr Ala Ala Arg Ala Gly
1410 1415 1420

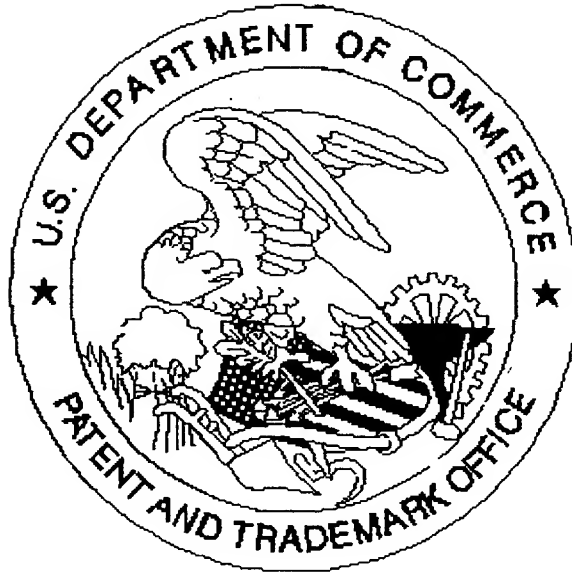
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His Leu Val Asn Gly Arg Met Asp Phe Ala Phe Pro Gly Ser Thr Asn
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Ser Leu His Arg Met Thr Thr Thr Ser Ala Ala Ala Tyr Gly Thr His
1460 1465 1470

Leu Ser Pro His Val Pro His Arg Val Leu Ser Thr Ser Ser Thr Leu
 1475 1480 1485
 Thr Arg Asp Tyr Asn Ser Leu Thr Arg Ser Glu His Ser His Ser Thr
 1490 1495 1500
 Thr Leu Pro Arg Asp Tyr Ser Thr Leu Thr Ser Val Ser Ser His Gly
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 Ala Leu Gly Ser Arg Ser Arg Ala Gln Met Lys Gly Phe Pro Pro Ser
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 Arg Gly Pro Arg Asp Ser Ile Ile Leu Ala Gly Arg Pro Ala Ala Pro
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 Trp Gln Glu Pro Arg Cys Glu Arg Pro Leu Gln Gly Tyr Ser Val Glu
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 Tyr Gln Leu Leu Asn Gly Gly Glu Leu His Arg Leu Asn Ile Pro Asn
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 Pro Ala Gln Thr Ser Val Val Val Glu Asp Leu Leu Pro Asn His Ser
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 Tyr Val Phe Arg Val Arg Ala Gln Ser Gln Glu Gly Trp Gly Arg Glu
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 1780 1785 1790

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